

Cotransfection of 293Cre cells with pBHG10lox and
a "Lox" shuttle plasmid for generation of Ad expression vectors

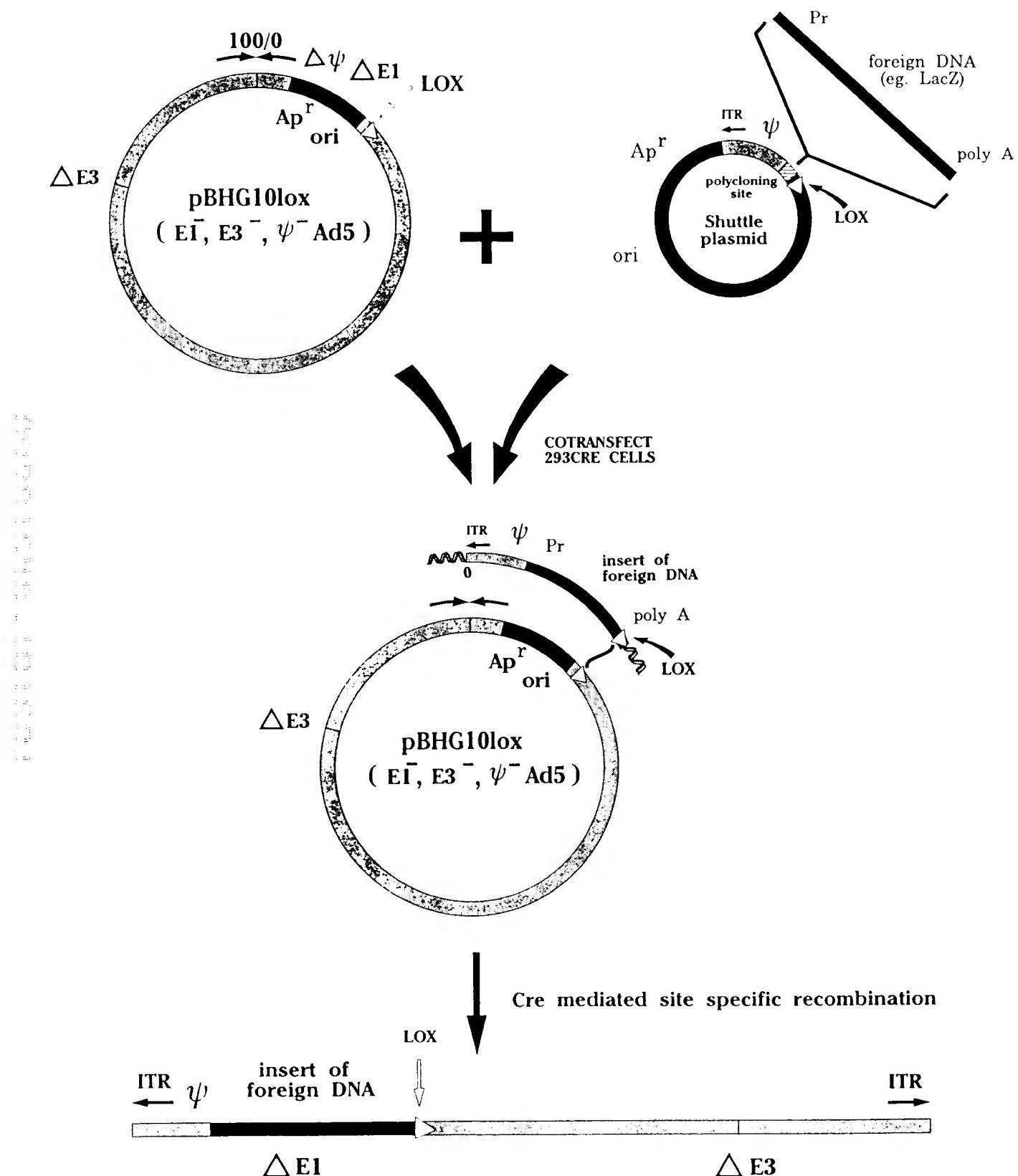


Fig. 1

Cotransfection of 293Cre cells with pBHG10lox and a "lox" shuttle plasmid for generation of Ad expression vectors

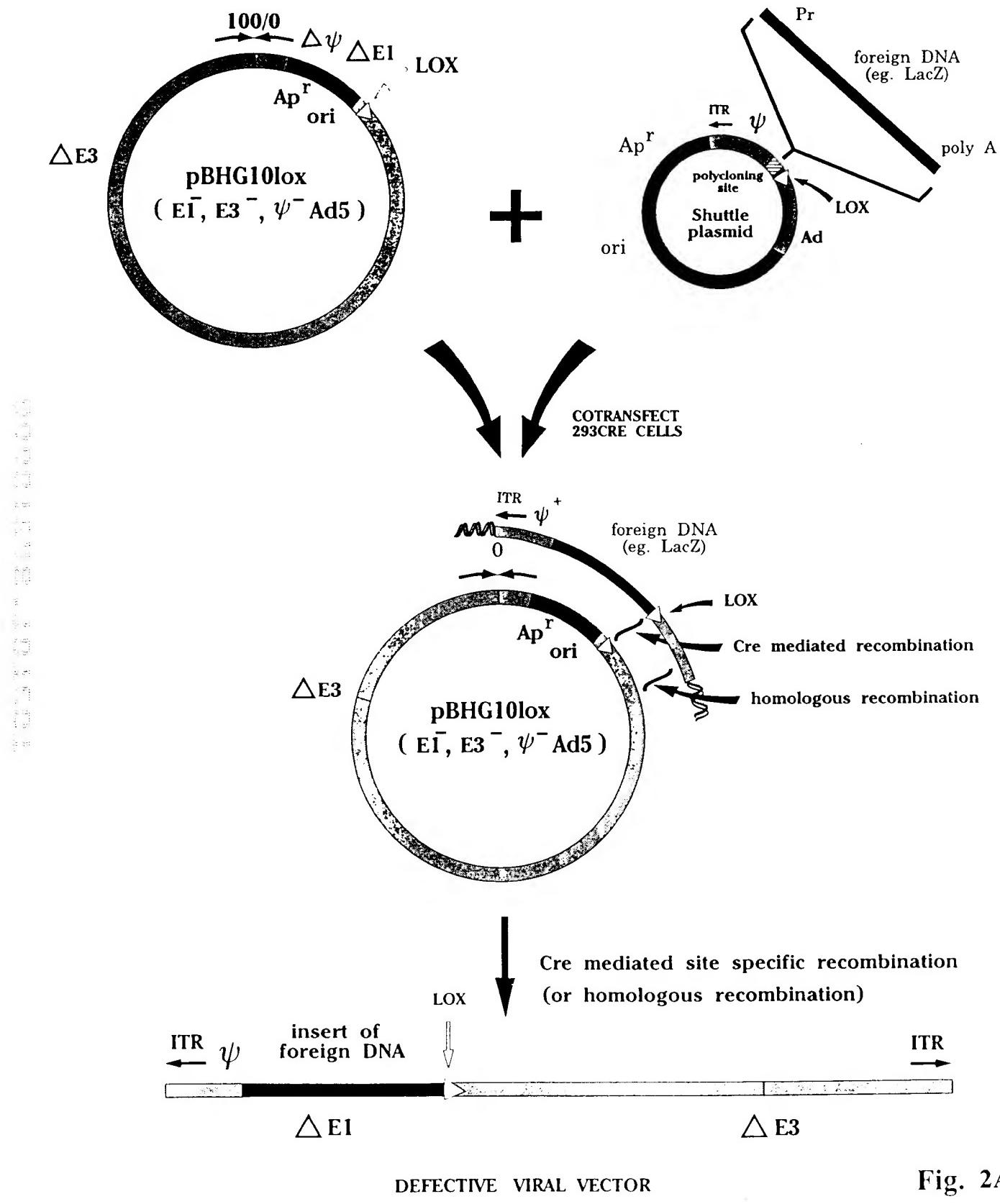


Fig. 2A

CONSTRUCTION OF VARIOUS SHUTTLE PLASMIDS

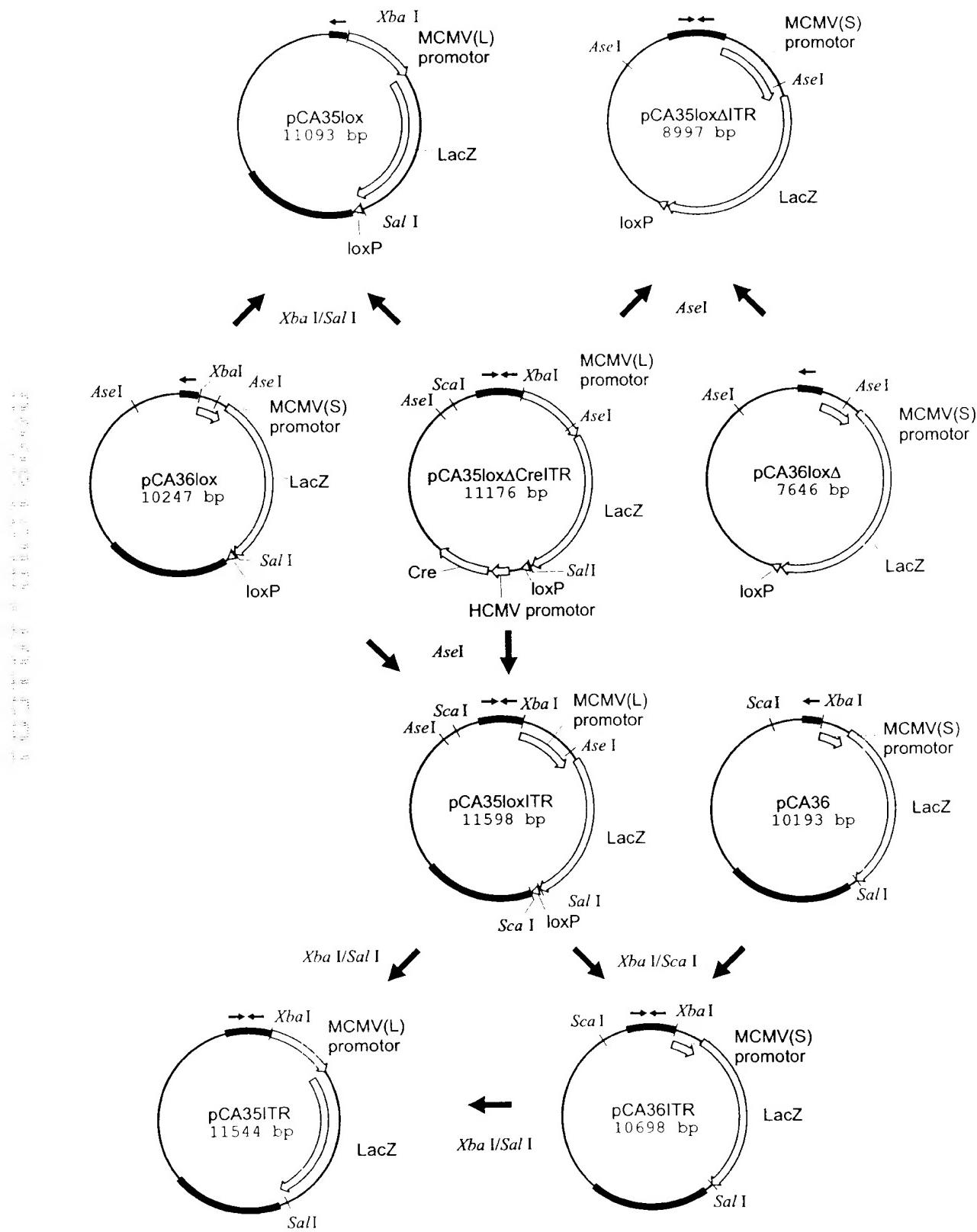


Figure 2B

OLIGONUCLEOTIDES USED IN CLONING

AB3233/3234 : loxP linker

SEQ. ID. NO. : 1

loxP site

5' GATCCAATAACTCGTATAGCATACATTATACGAAGTTATAAGTACTGAATTG 3'
 3' GTTATTGAAGCATATCGTATGTAATATGCTCAATATTGACTTAAGCCTAG 5'

BamH I/Bgl II
overhang

SEQ. ID. NO. : 2

BamH I/Bgl II
overhang

Sca I *EcoR I*

↓

SEQ. ID. NO. : 3

5' AATTCCCCGGGAGATCTAAGCTTGAGCTCG 3'
 3' GGGGCCCTCTAGATTGAACTCGAGCAGCT 5'

EcoR I overhang

SEQ. ID. NO. : 4

Sal I overhang

SEQ. ID. NO. : 5

AB6920/6921 : loxP linker

SEQ. ID. NO. : 6

Xba I overhang

5' CTAGCAATAACTCGTATAGCATACATTATACGAAGTTATATCGATG 3'
 3' GTTATTGAAGCATATCGTATGTAATATGCTCAATATAGCTACGATC 5'

Xba I overhang

SEQ. ID. NO. : 7

Cla I

Bpu I overhang

SEQ. ID. NO. : 8

5' TGACAATAACTCGTATAGCATACATTATACGAAGTTATATCGATG 3'

3' GTTATTGAAGCATATCGTATGTAATATGCTCAATATAGCTACACT 5'

Bpu I overhang

SEQ. ID. NO. : 8

Cla I

Fig. 3

CONSTRUCTION OF A CIRCULAR GENOMIC PLASMID FOR Ad VECTOR RESCUE USING THE Cre/ loxP SYSTEM

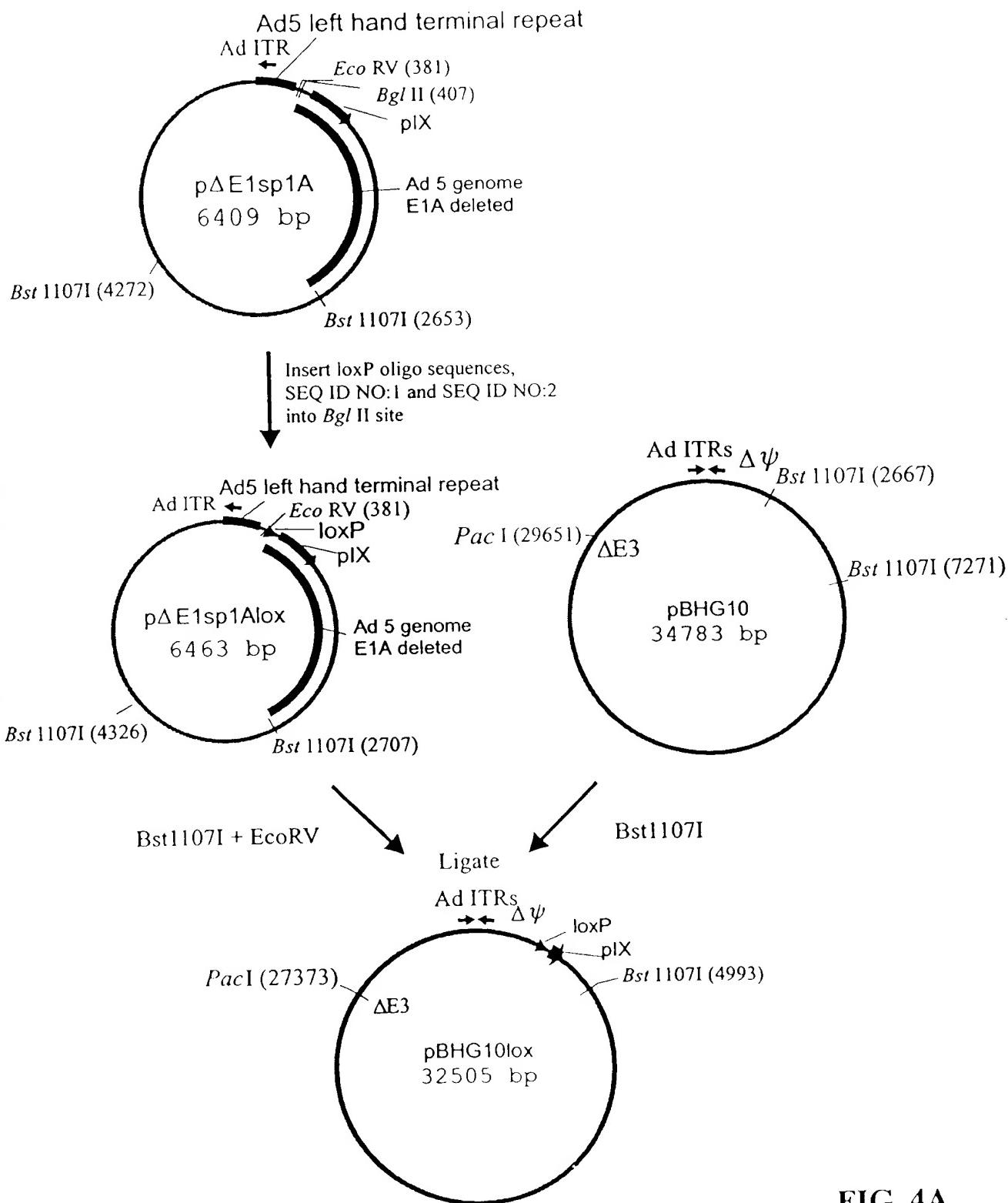


FIG. 4A

CONSTRUCTION OF pBHGdX1P_{lox}

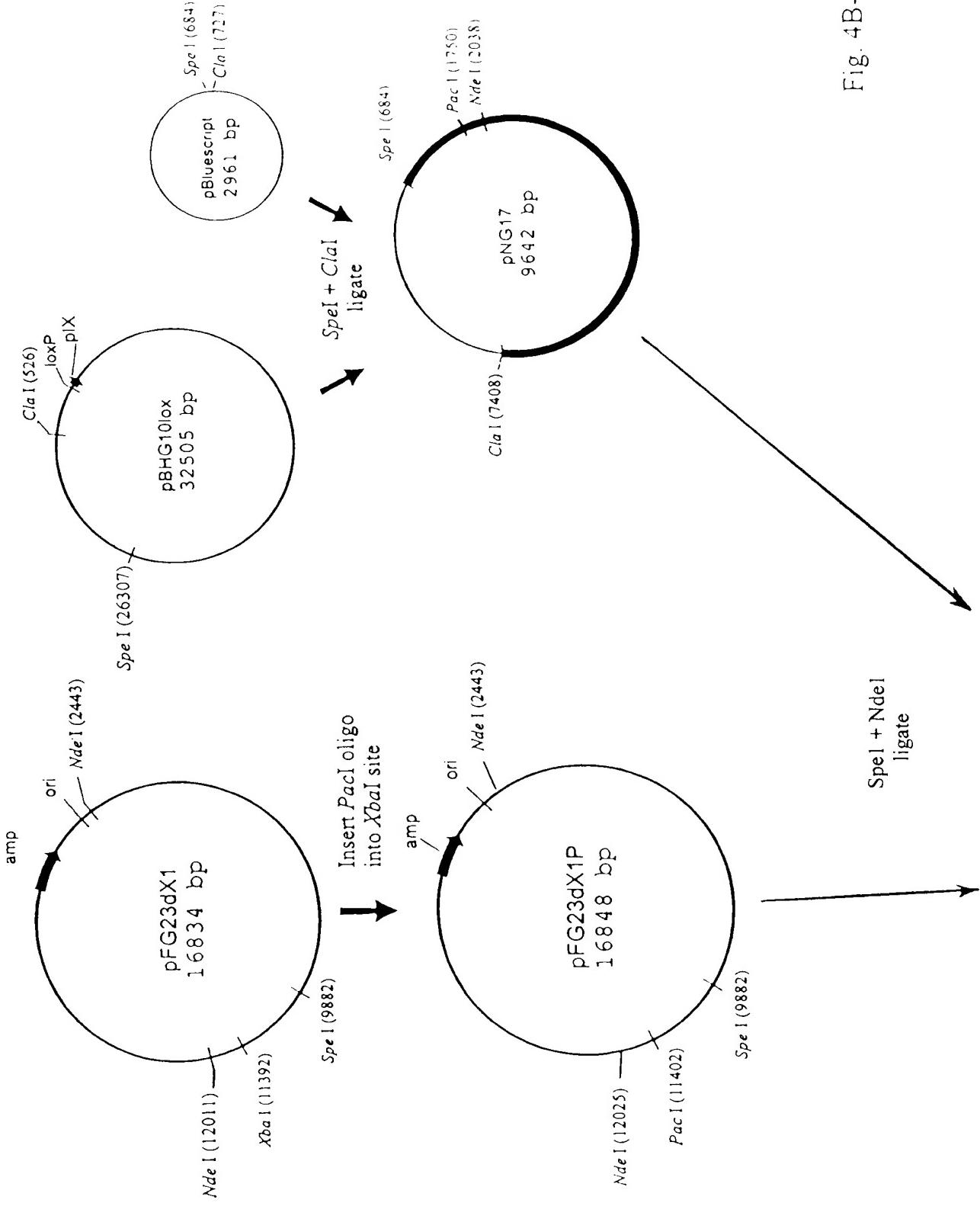


Fig. 4B-1

CONSTRUCTION OF pBHGdX1P_{lox}

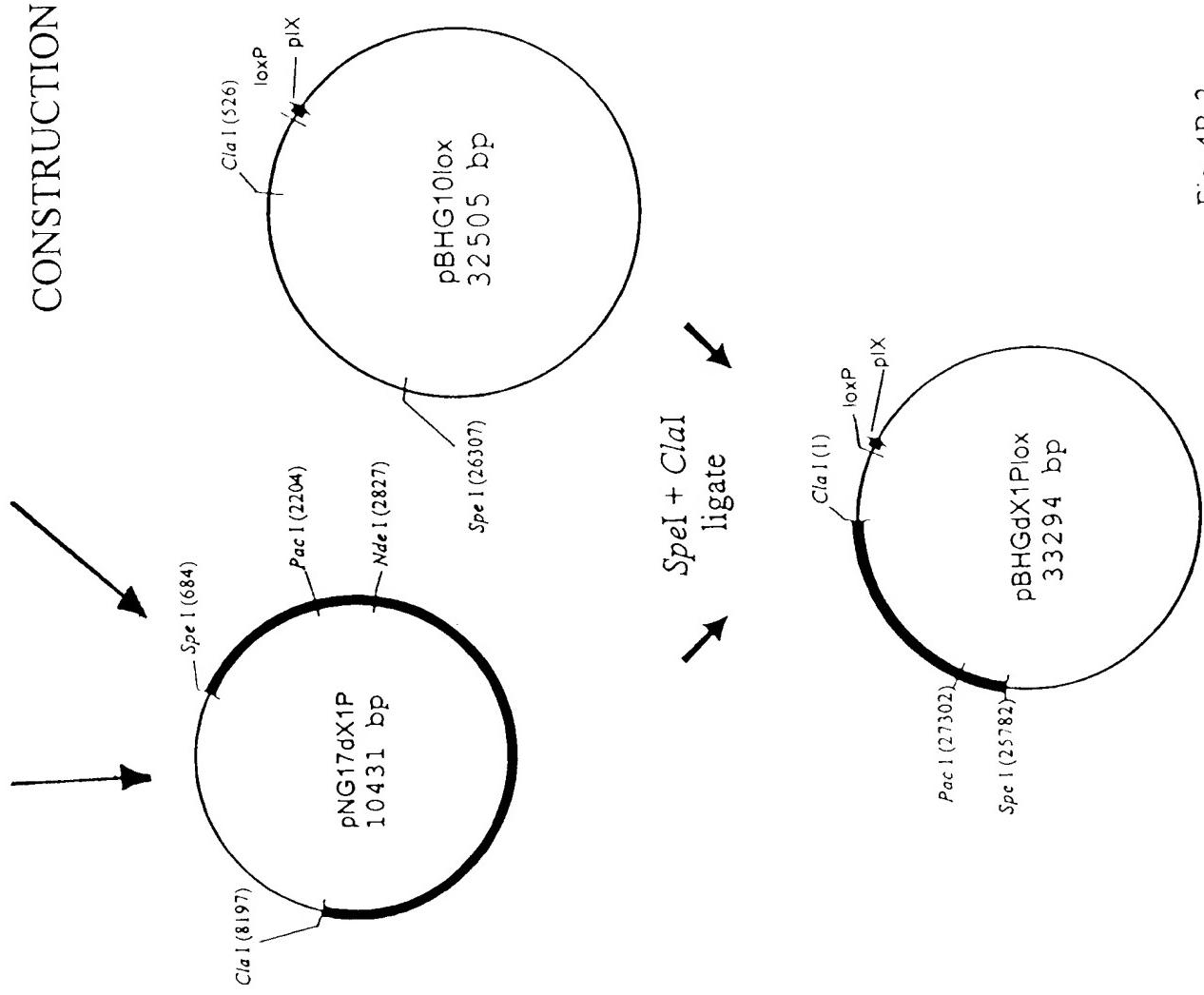


Fig. 4B-2

CONSTRUCTION OF pBHGE3lox

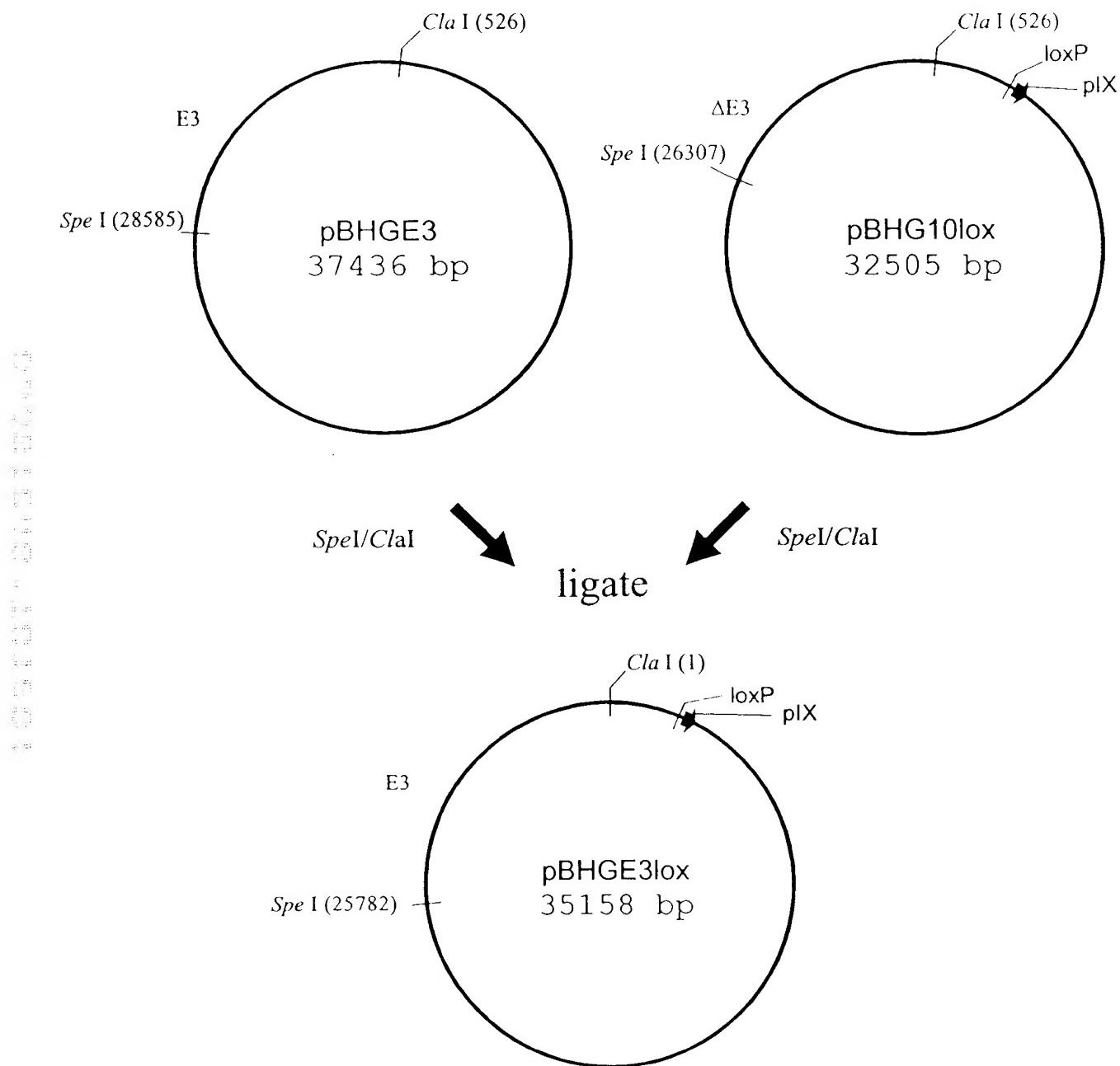


Fig. 4C

CONSTRUCTION OF Ad GENOMIC PLASMIDS ENCODING CRE

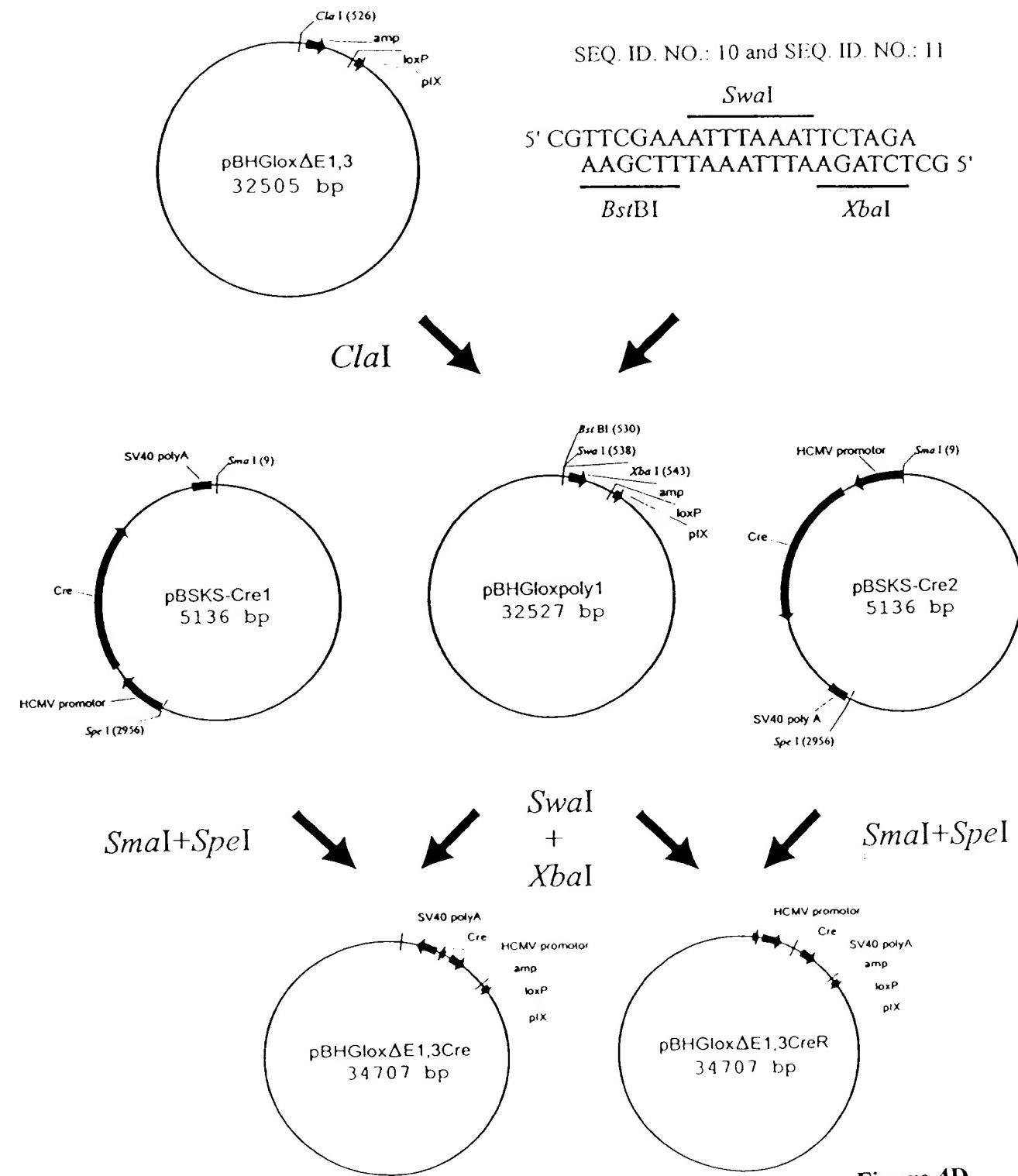


Figure 4D

CONSTRUCTION OF pΔE1SP1A & pΔE1SP1B loxP PLASMIDS FOR RESCUE OF FOREIGN DNA

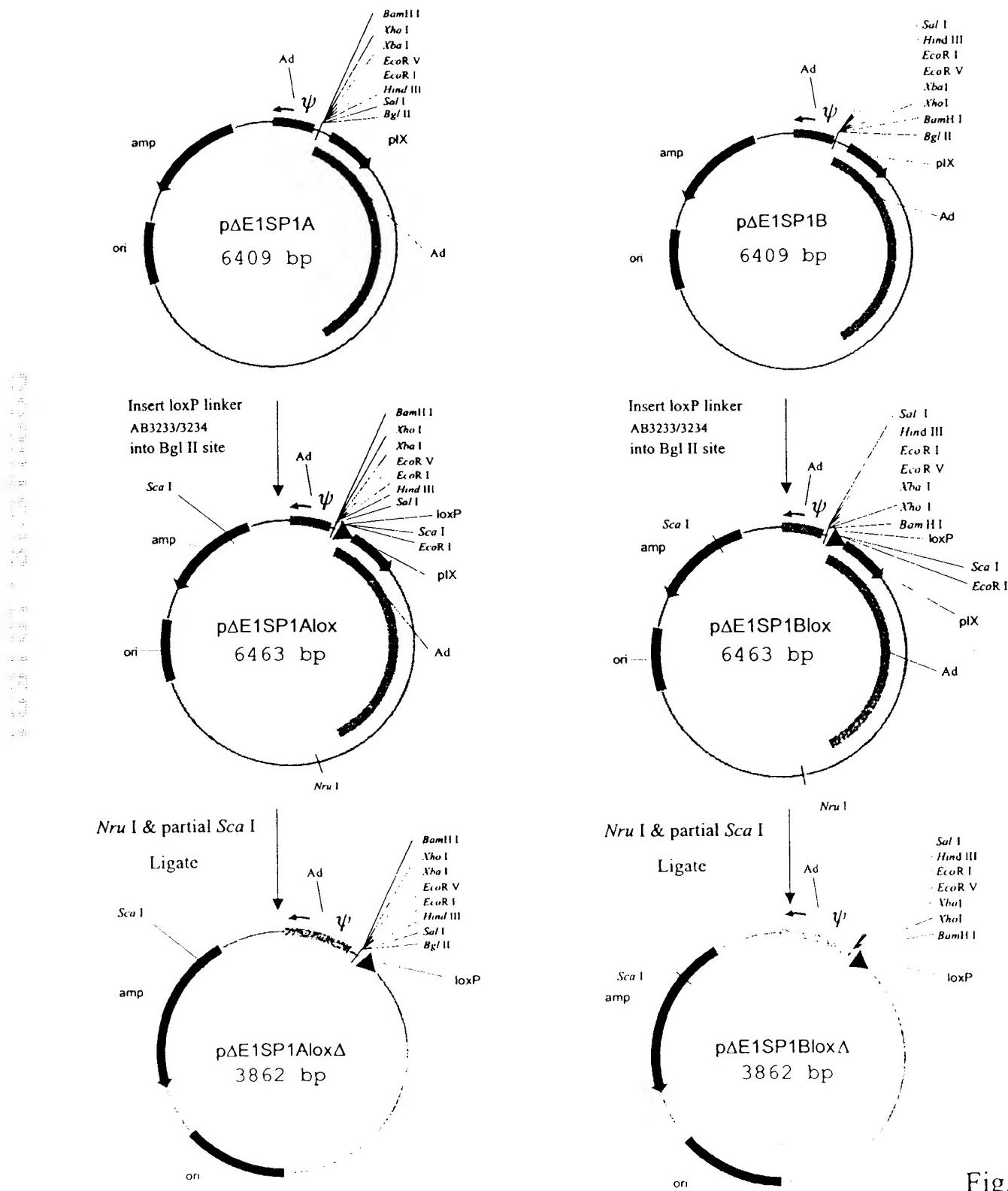


Fig. 5A

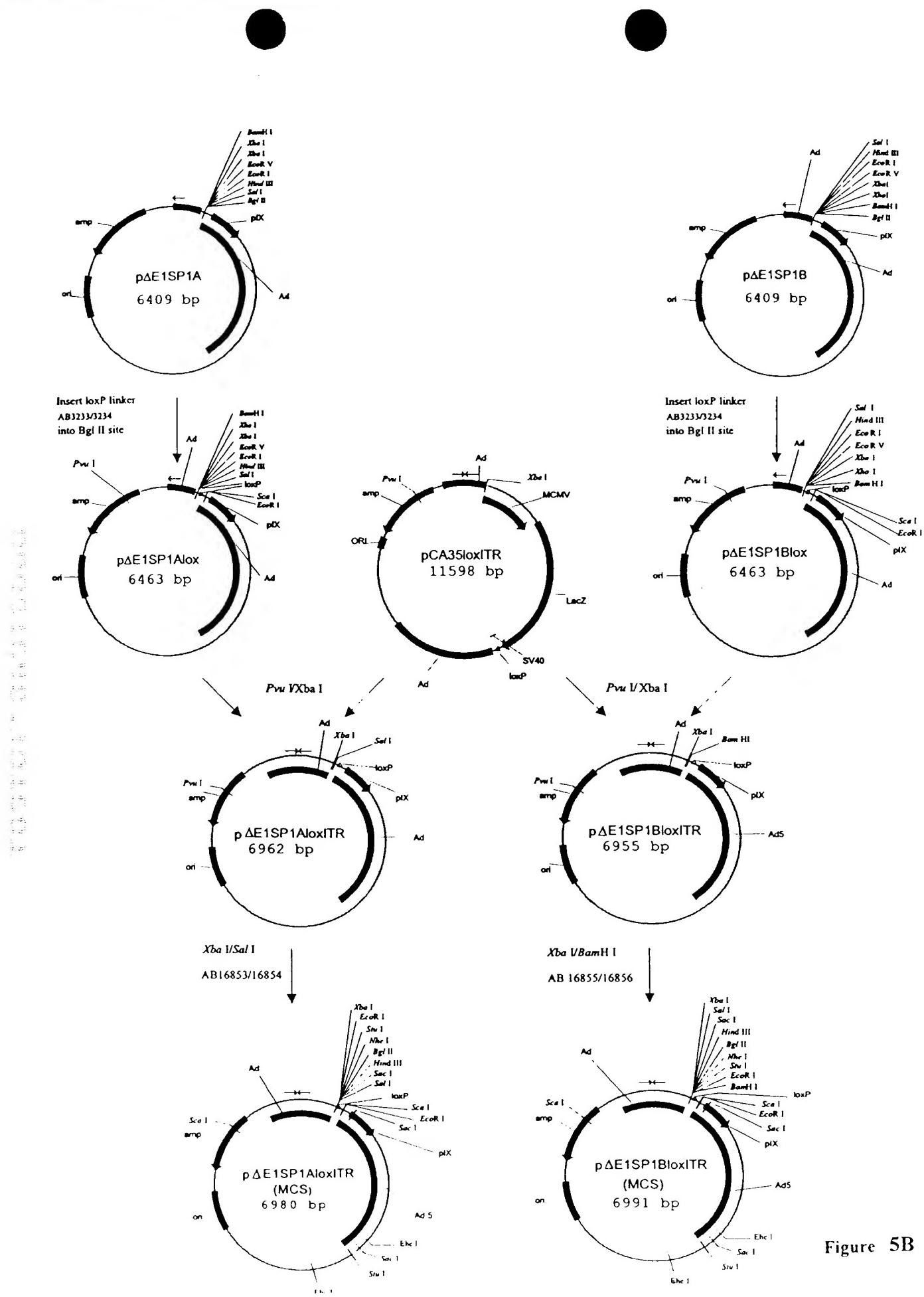


Figure 5B

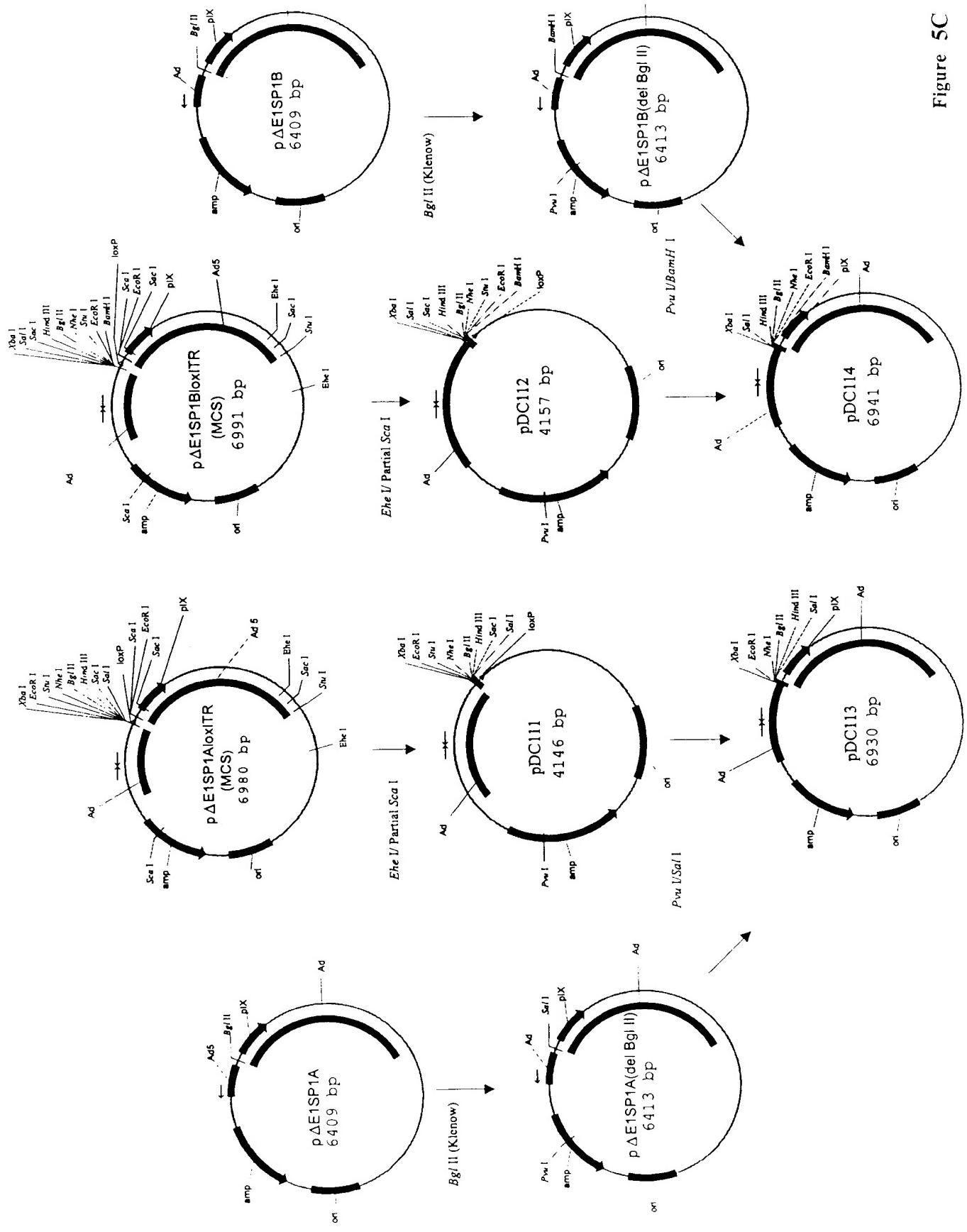
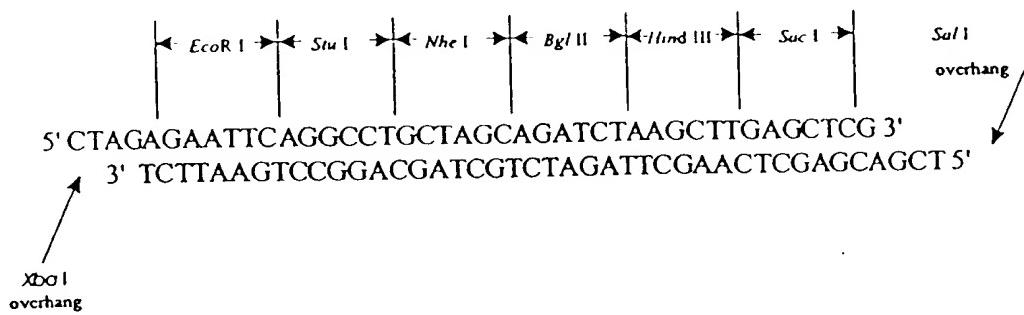


Figure 5C

SEQ. ID. NO.: 12 (AB16853) and SEQ. ID. NO.: 13 (AB16854)



SEQ. ID. NO.: 14 (AB16855) and SEQ. ID. NO.: 15 (AB16856)

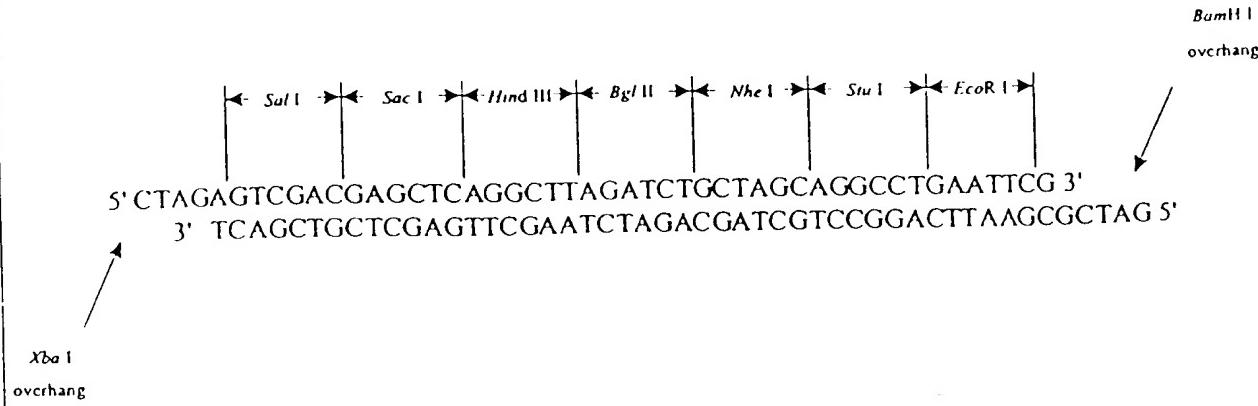


Figure 5D

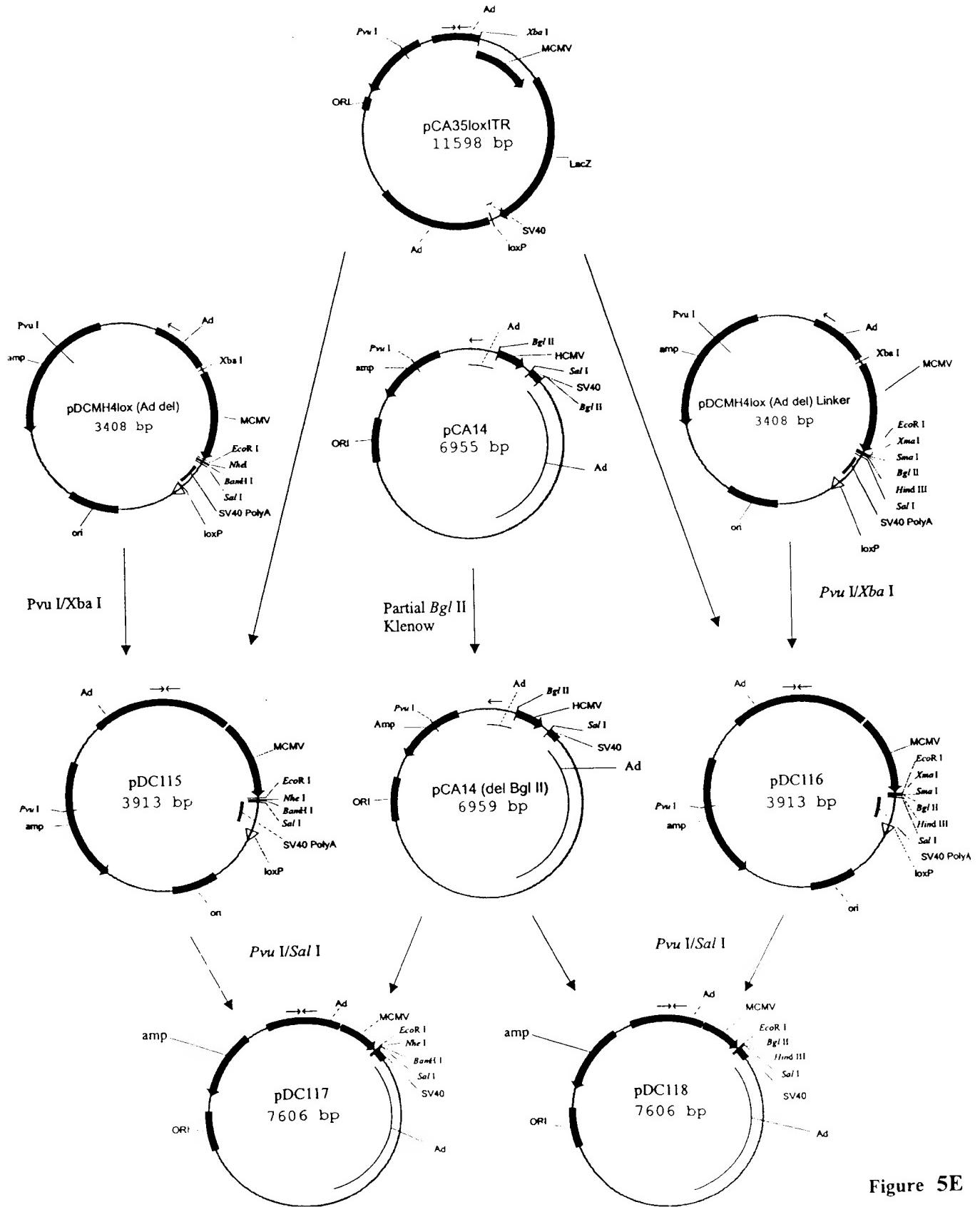


Figure 5E

CONSTRUCTION OF pMH4LOX, pMH4LOX Δ and pMH4LOX Δ LINK SHUTTLE PLASMIDS FOR RESCUE OF EXPRESSION CASSETTES

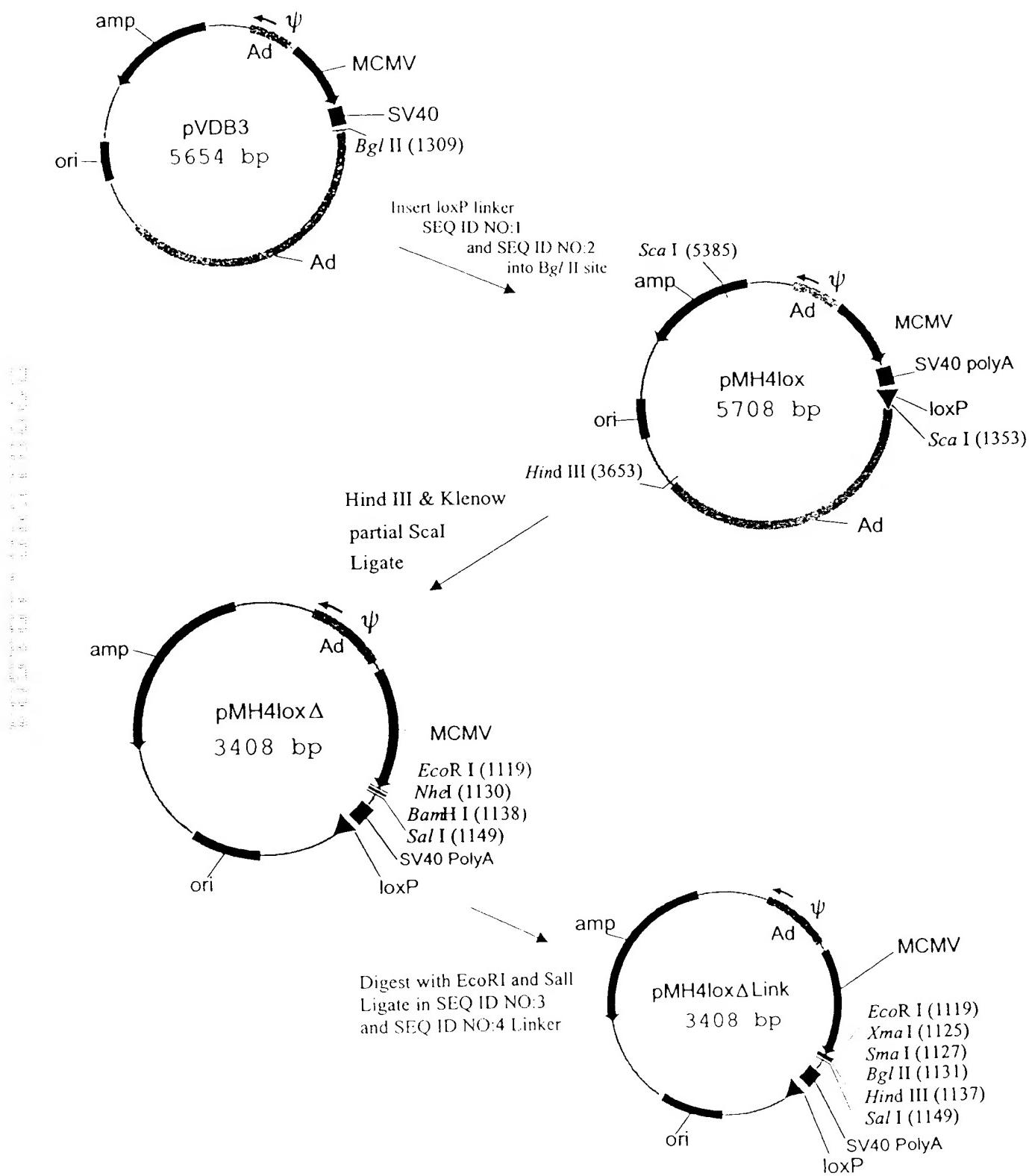


Fig. 6A

CONSTRUCTION OF A SHUTTLE PLASMID CONTAINING A pUC DERIVED ORIGIN

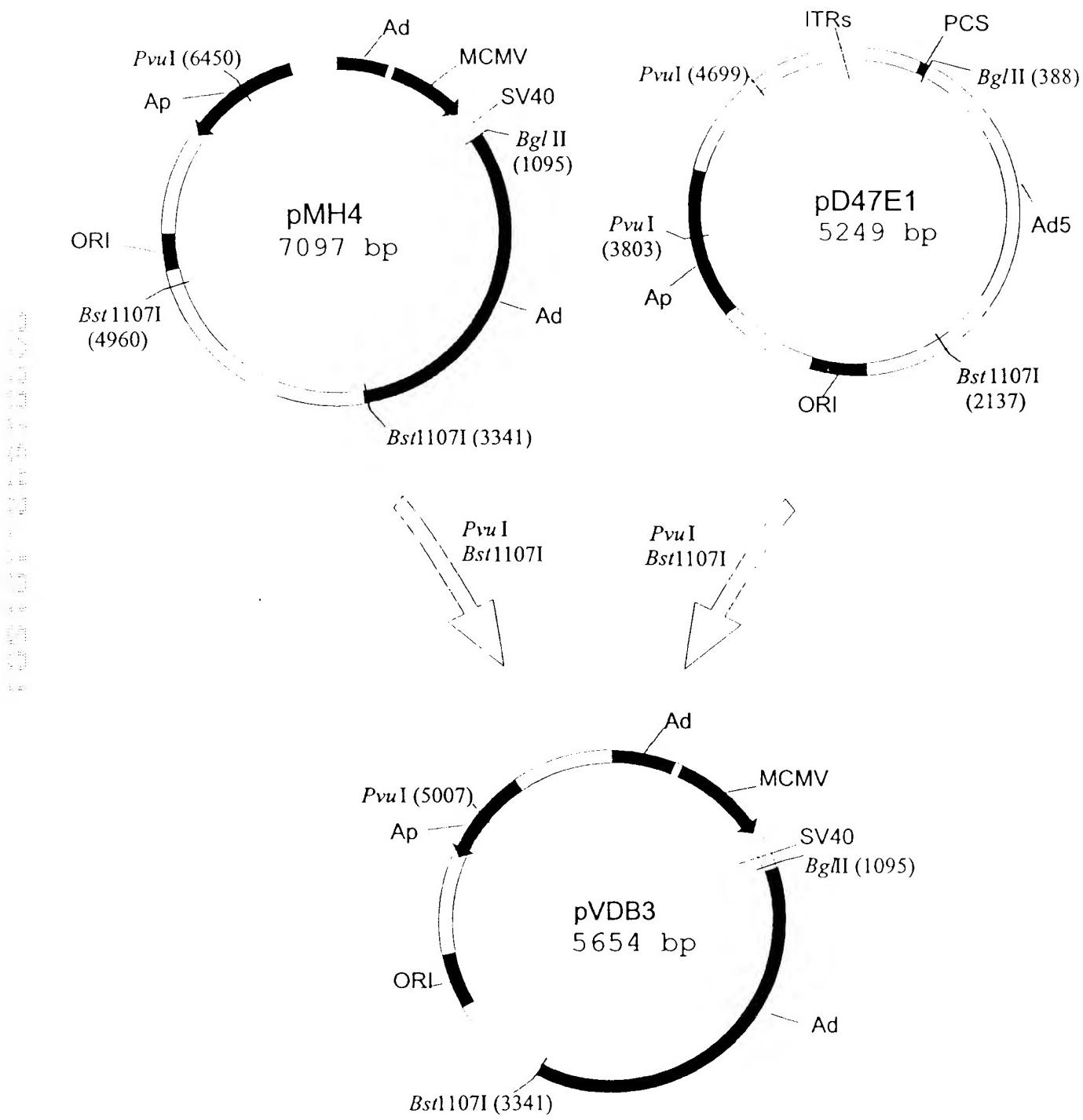


Fig. 6B

CONSTRUCTION OF HCMV loxP PLASMIDS FOR RESCUE OF EXPRESSION CASSETTES

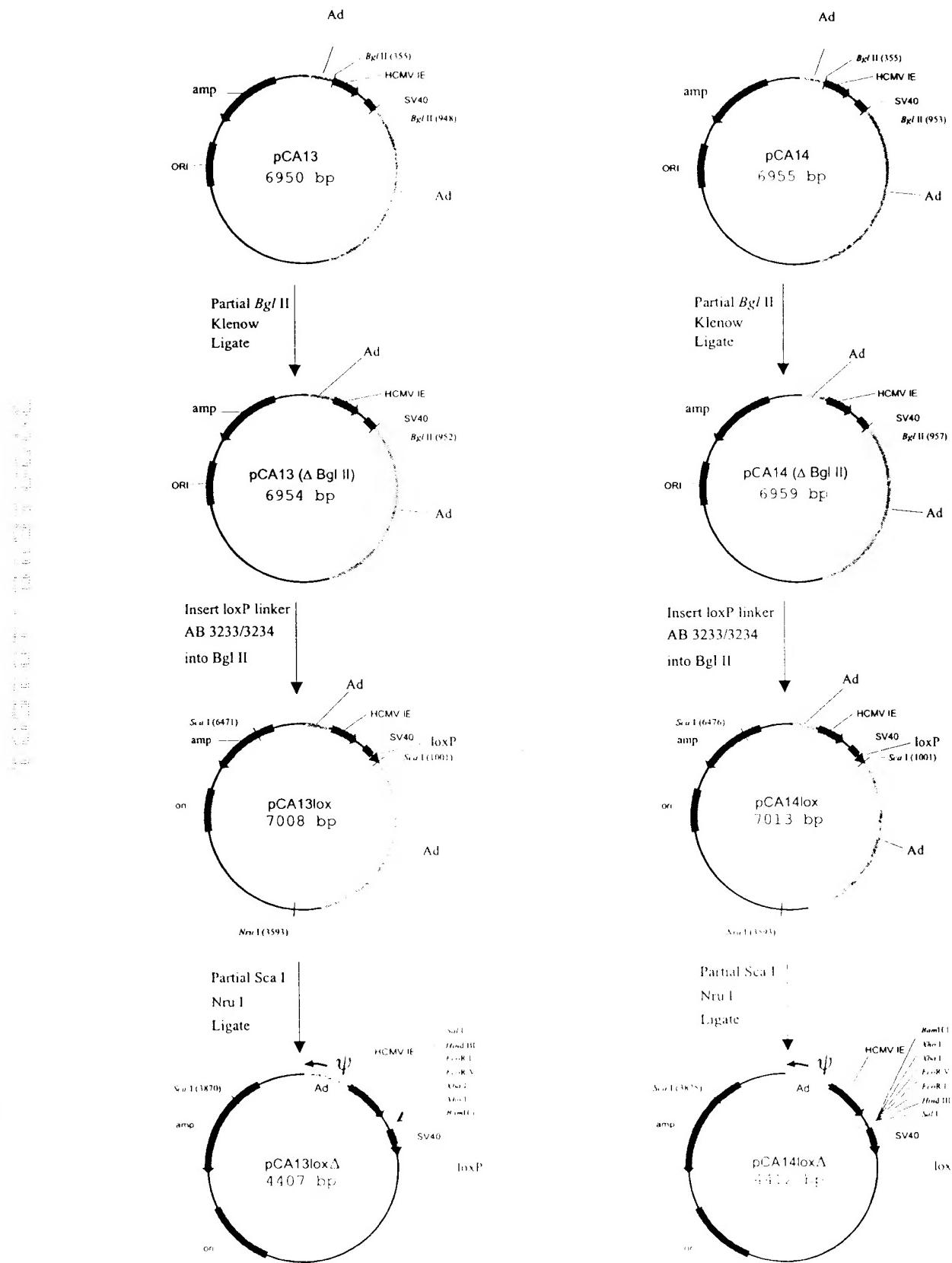


Fig. 7

CONSTRUCTION OF pCA36LOX and pCA36LOX Δ SHUTTLE PLASMIDS FOR RESCUE OF LACZ

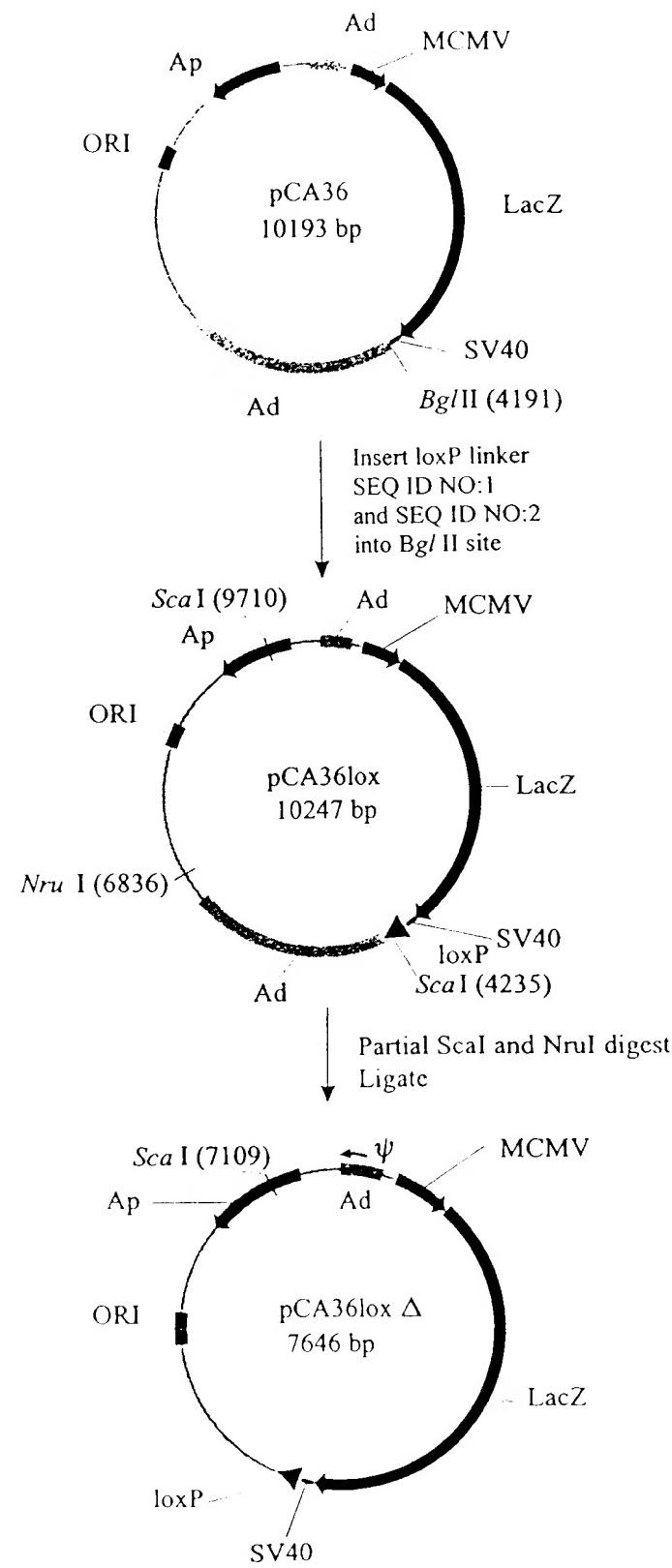


Fig. 8A

Cotransfection of 293Cre cells with AdLC8c DNA-TP and a shuttle plasmid containing a loxP site for generation of Ad expression vectors

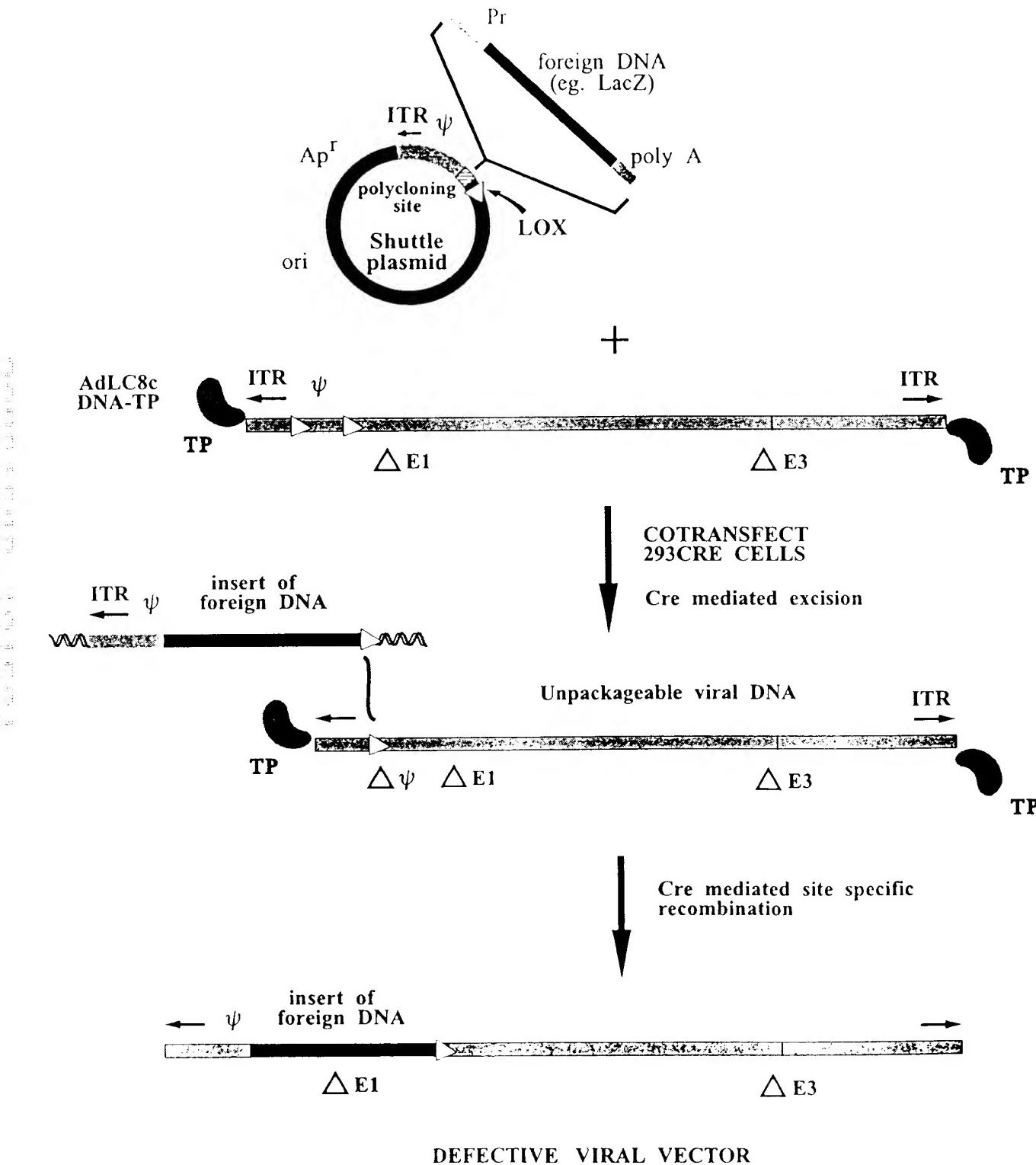


Fig. 8B

Cotransfection of 293Cre cells with restricted AdLC8c DNA-TP and loxP shuttle plasmid for generation of Ad expression vectors

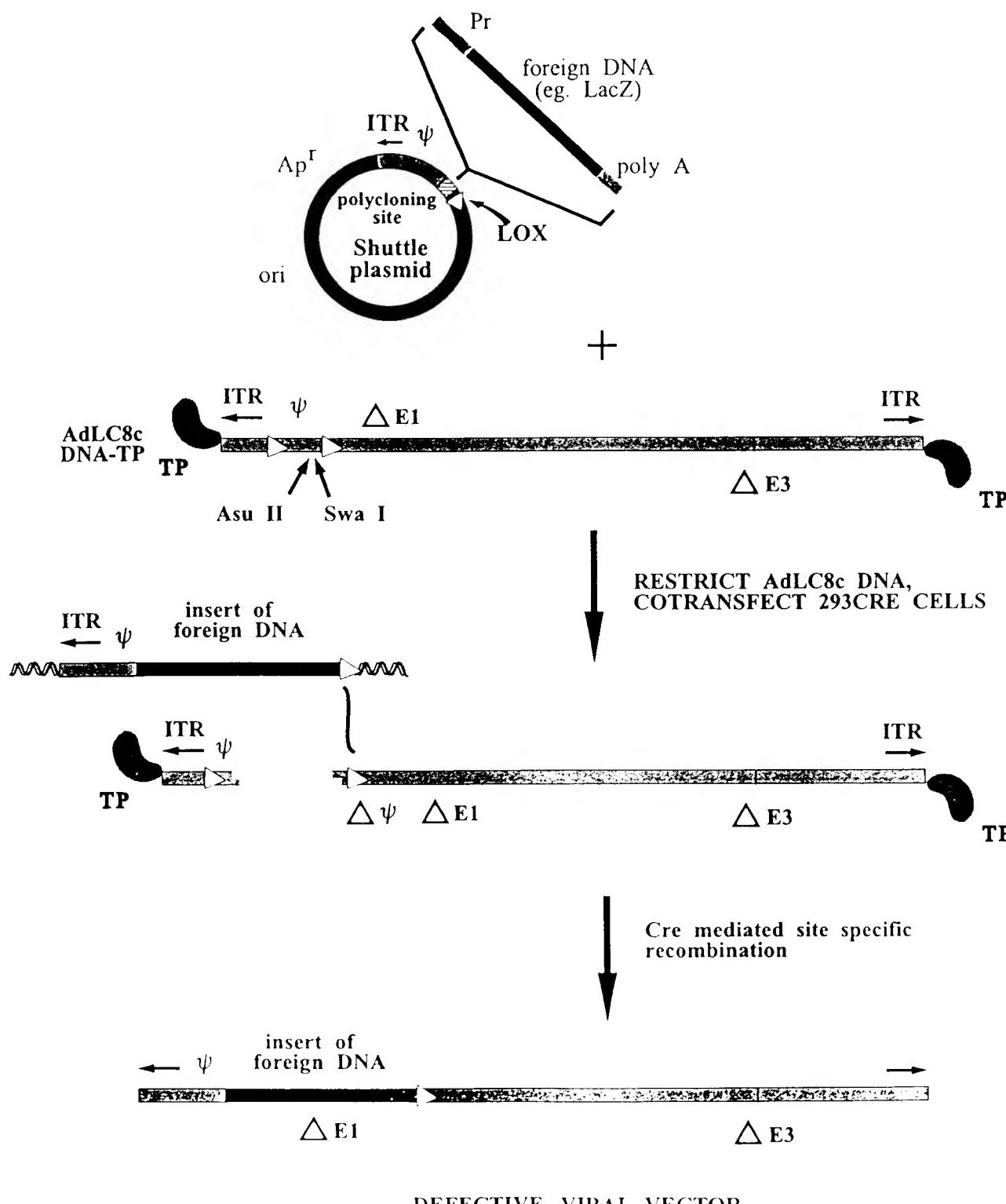


Fig. 8C

CONSTRUCTION OF SHUTTLE PLASMIDS EXPRESSING Cre

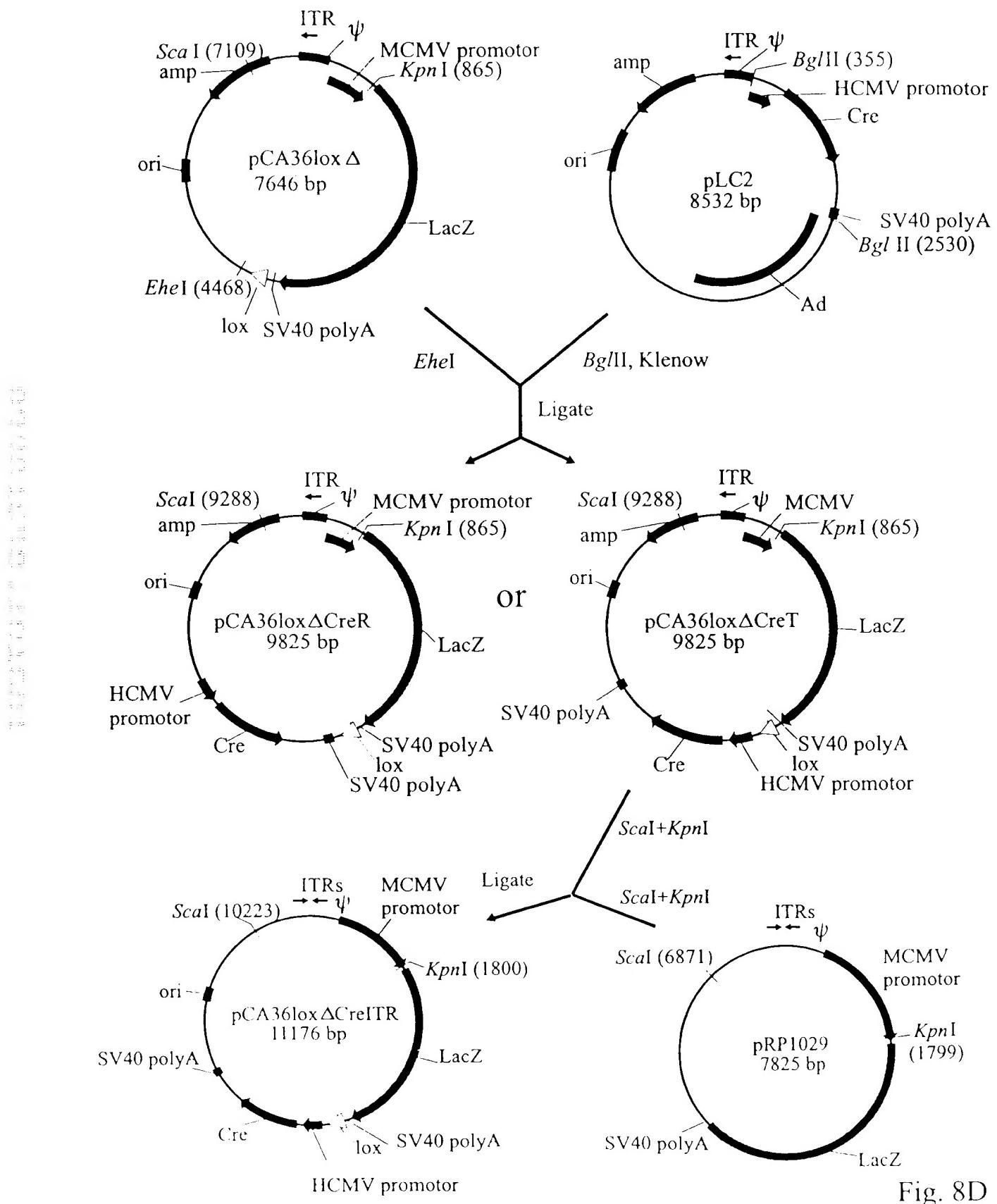


Fig. 8D

Cotransfection of 293 cells with pBHG10lox and a "Lox" shuttle plasmid expressing Cre for generation of Ad expression vectors

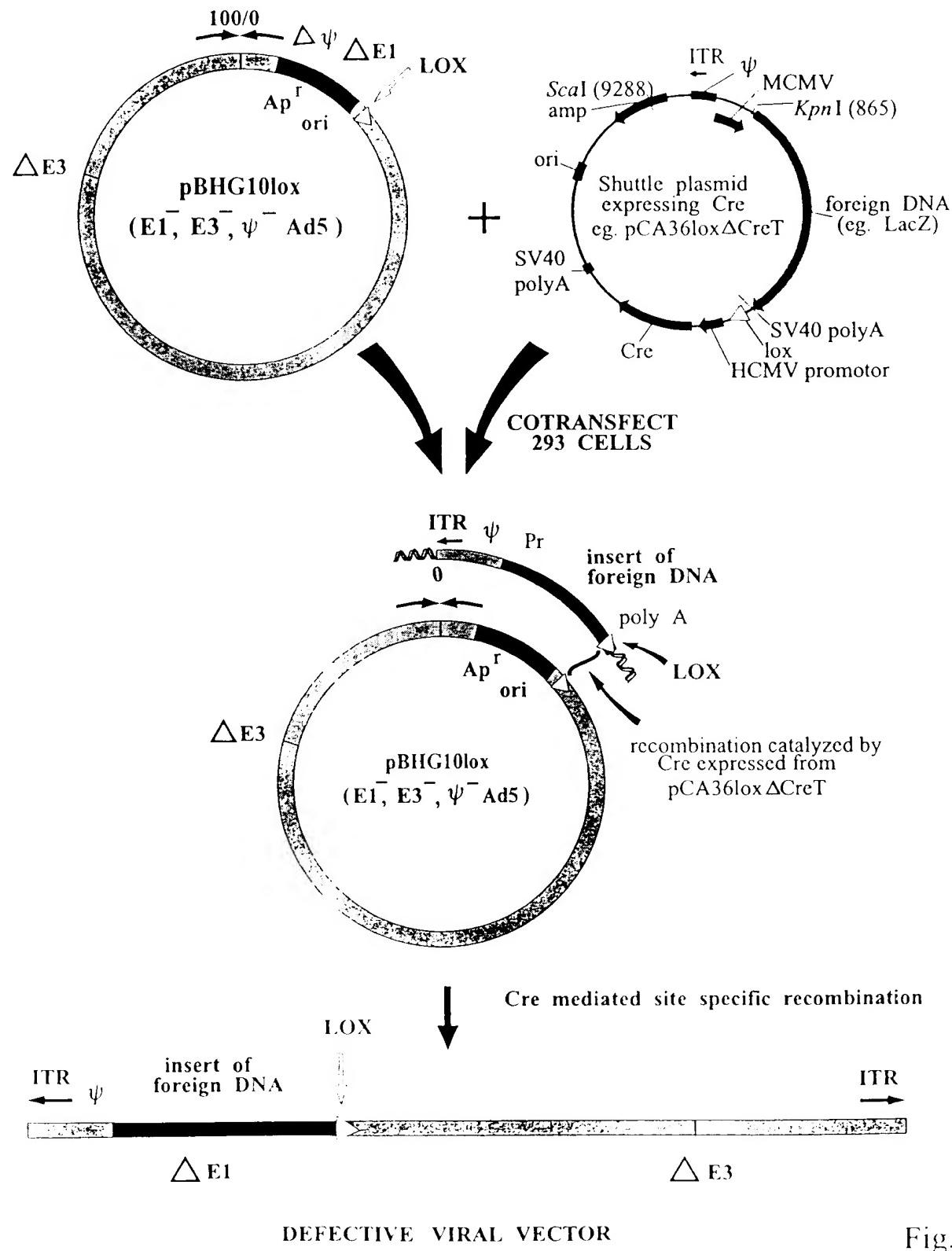


Fig. 8E

CONSTRUCTION OF Ad GENOMIC PLASMID ENCODING CRE

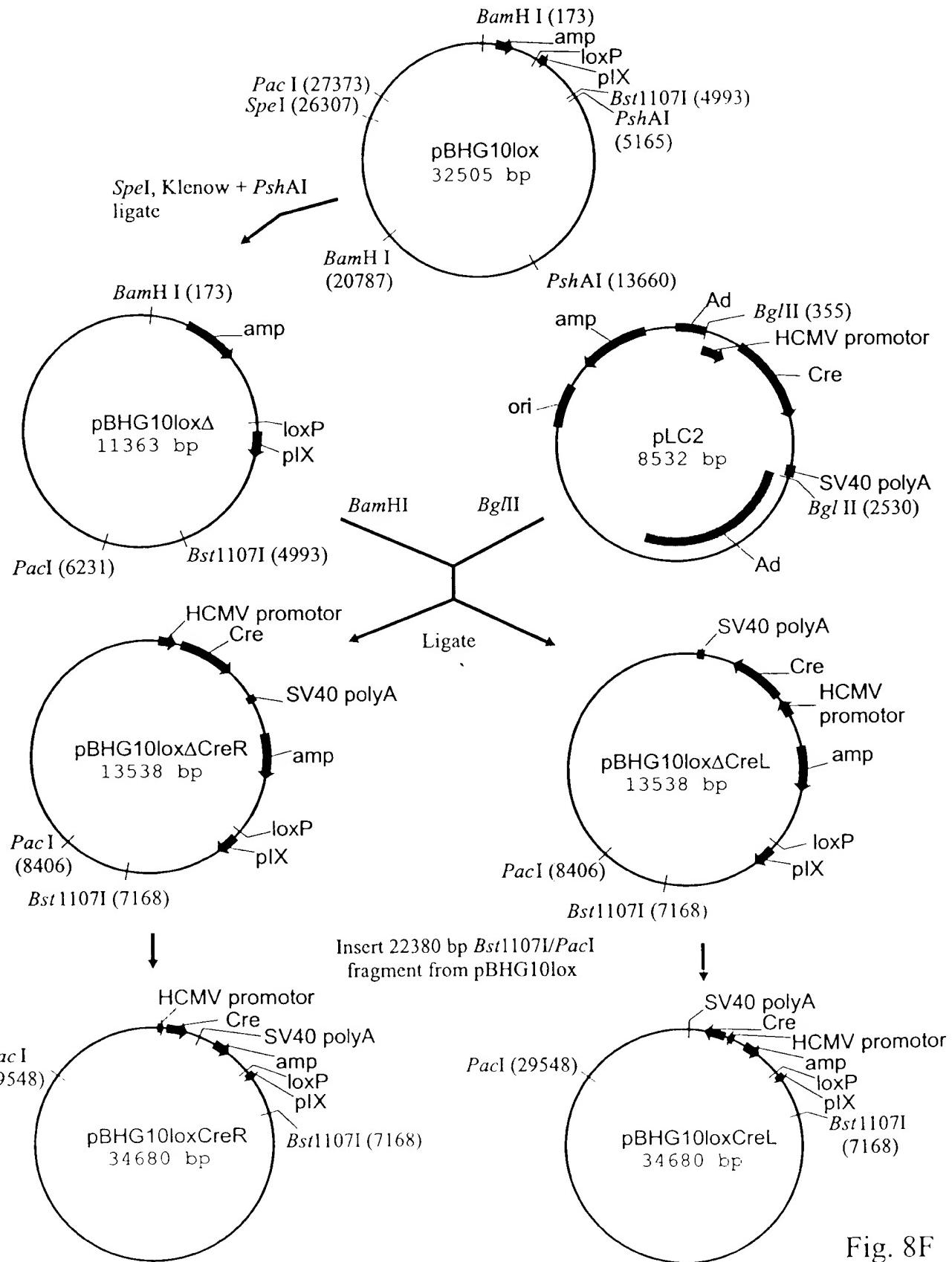


Fig. 8F

RESCUE OF FIBRE MUTATIONS USING CRE/LOX RECOMBINATION

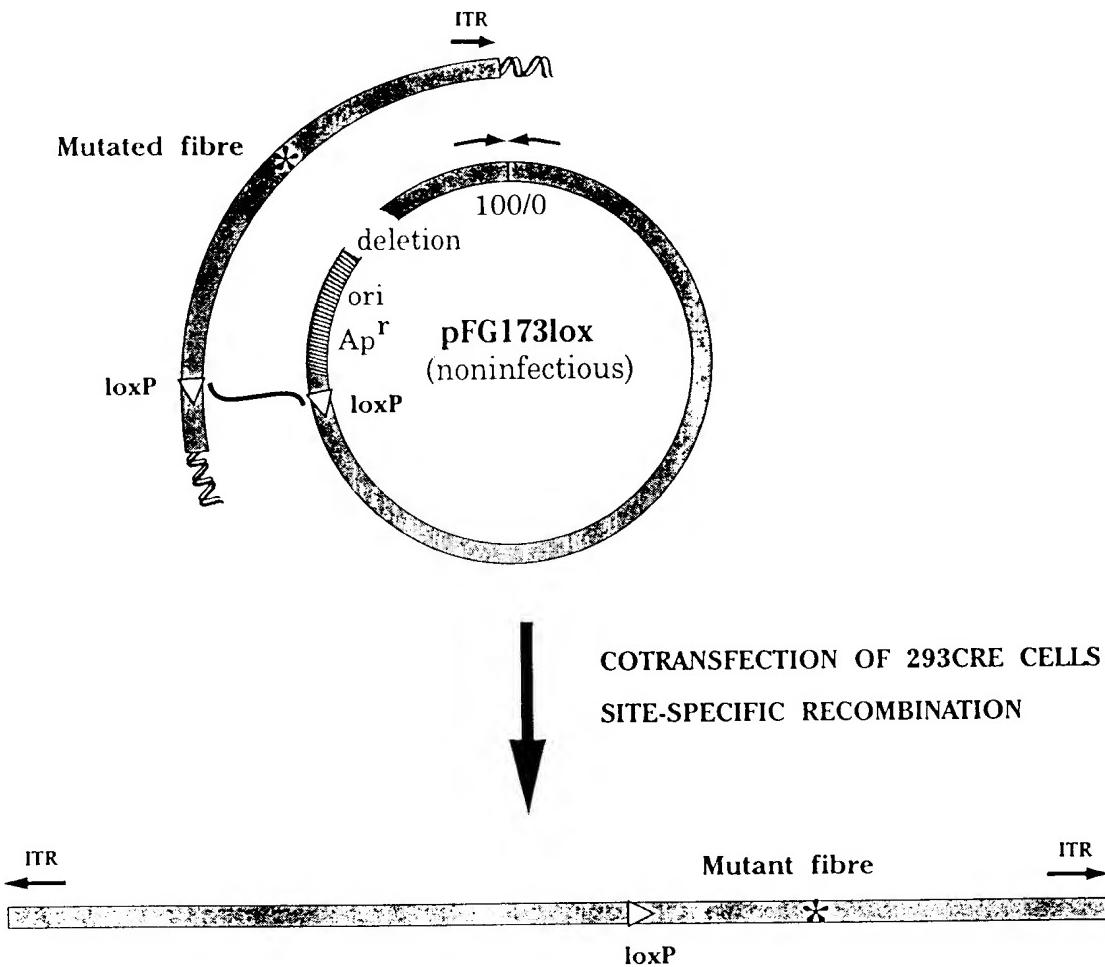


Fig. 9A

CONSTRUCTION OF pAB14lox Δ

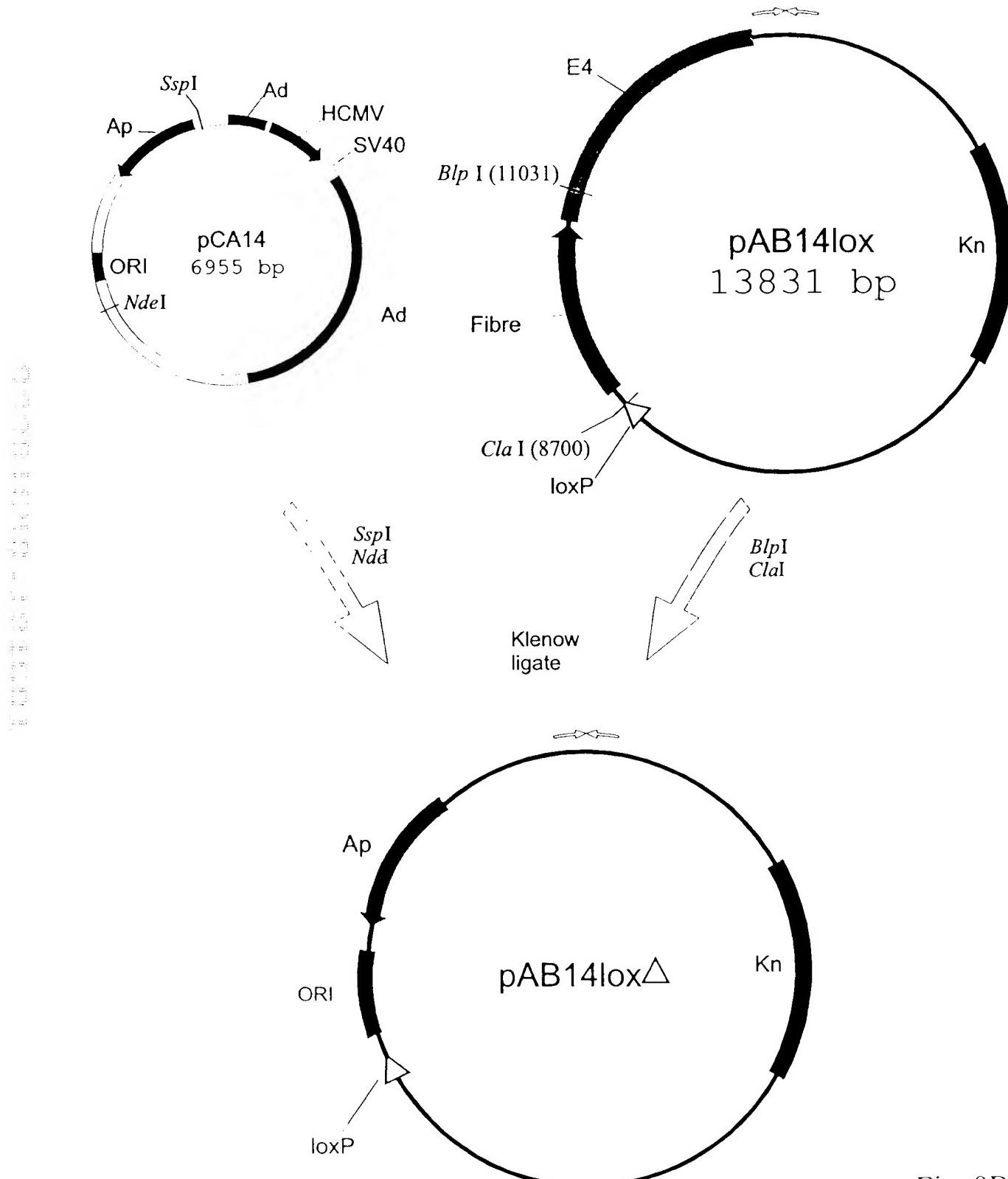
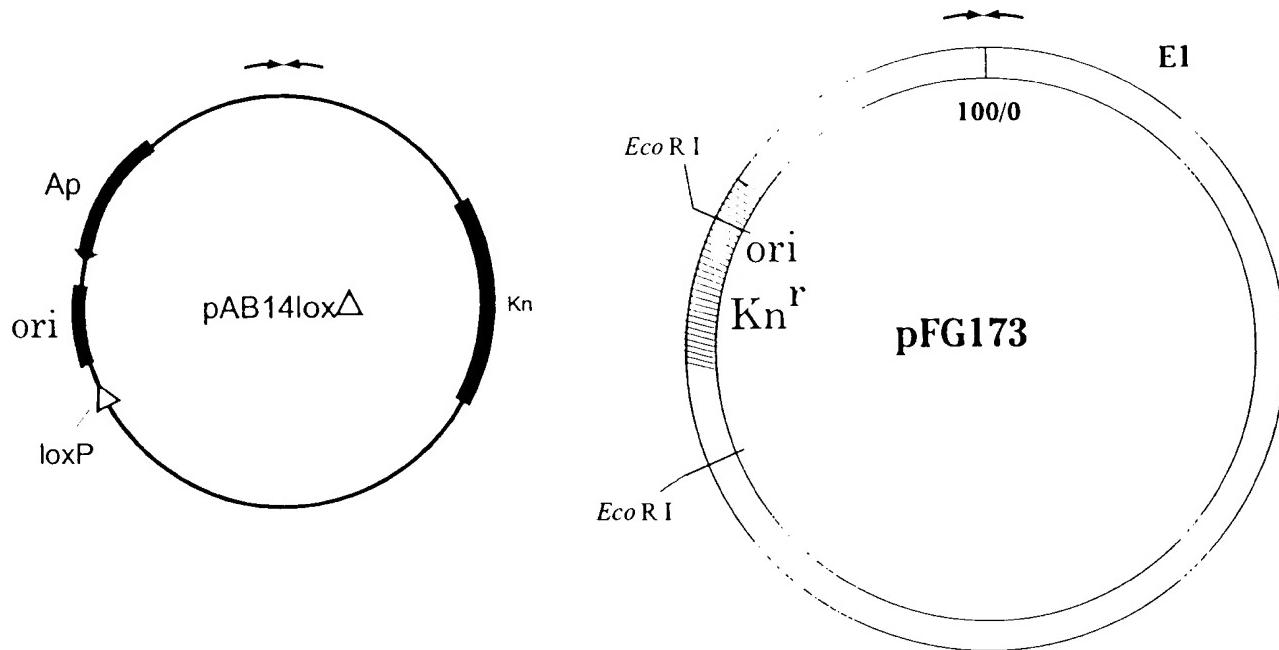


Fig. 9B

CONSTRUCTION OF pFG173lox



Restriction, transformation of *E. coli*,
homologous recombination

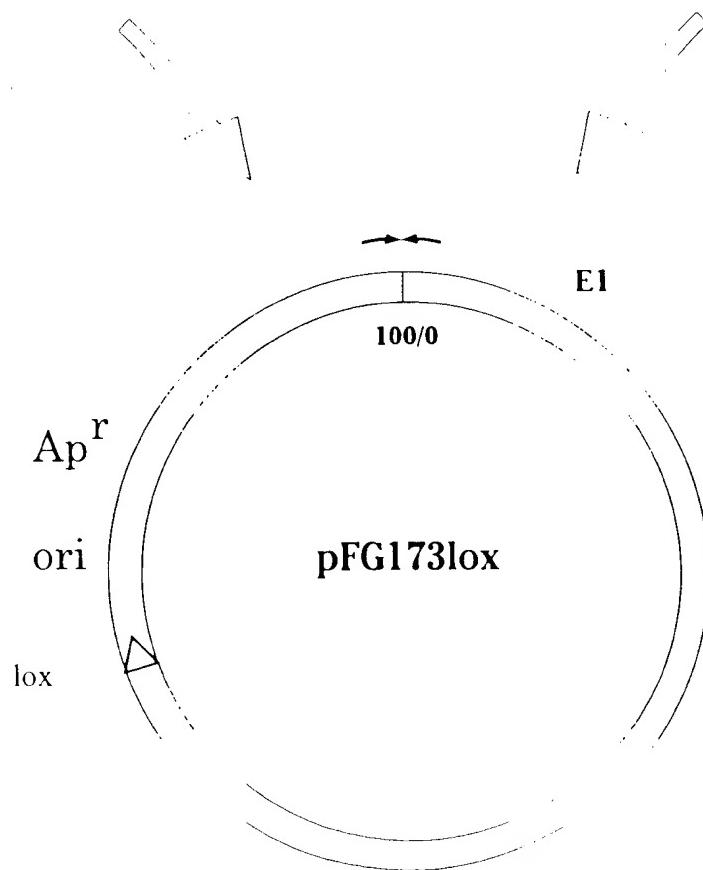


Fig. 9C

CONSTRUCTION OF pFG23dX1lox AND pFG23dX1lox_C FOR RESCUE OF MUTANT FIBRE INTO AD VIRUS

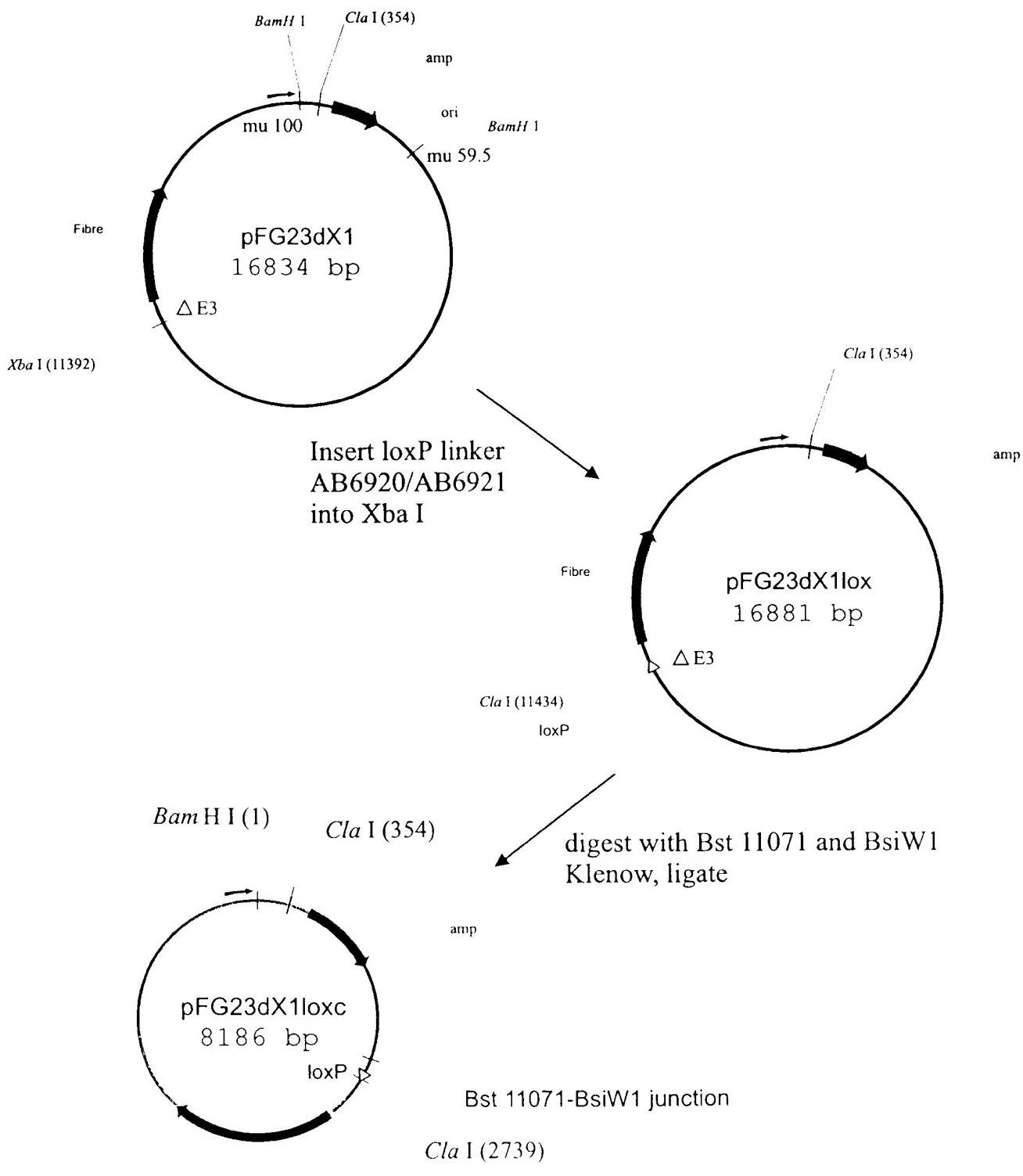


Fig. 10

A PLASMID FOR RESCUE OF A FOREIGN DNA INTO AD VIRUS

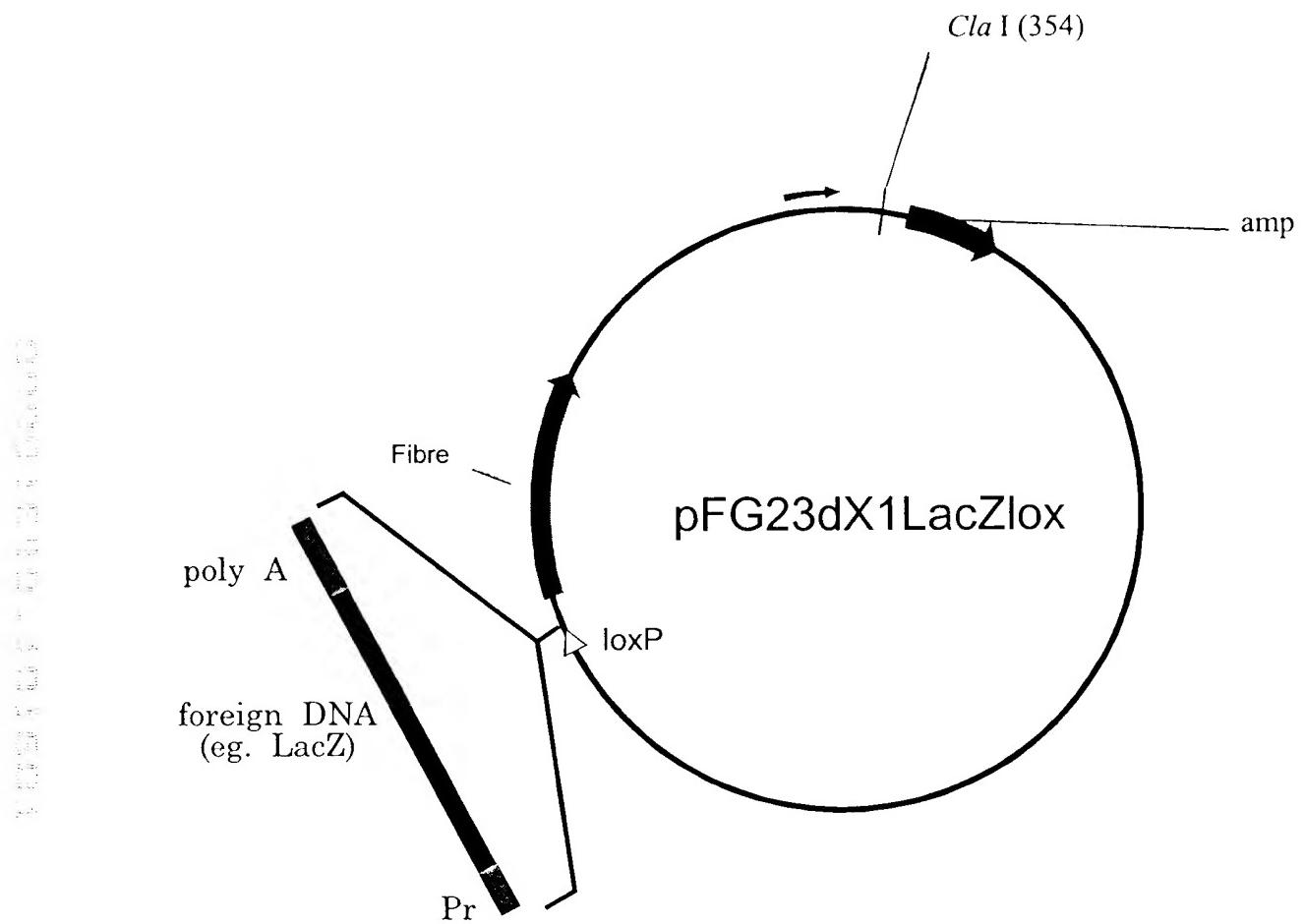


Fig. 11A

RESCUE OF FIBRE MUTATIONS USING CRE/LOX RECOMBINATION

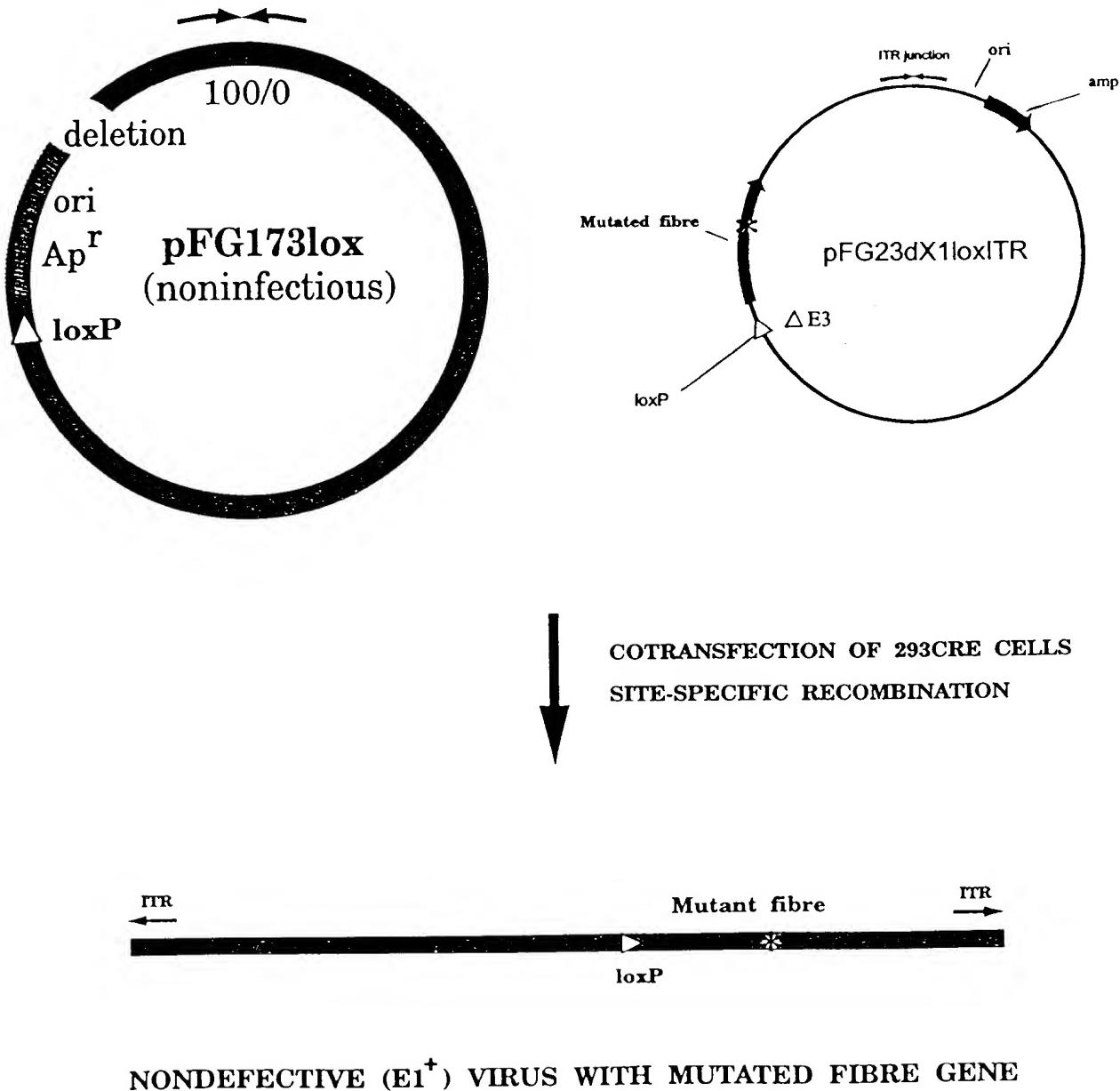
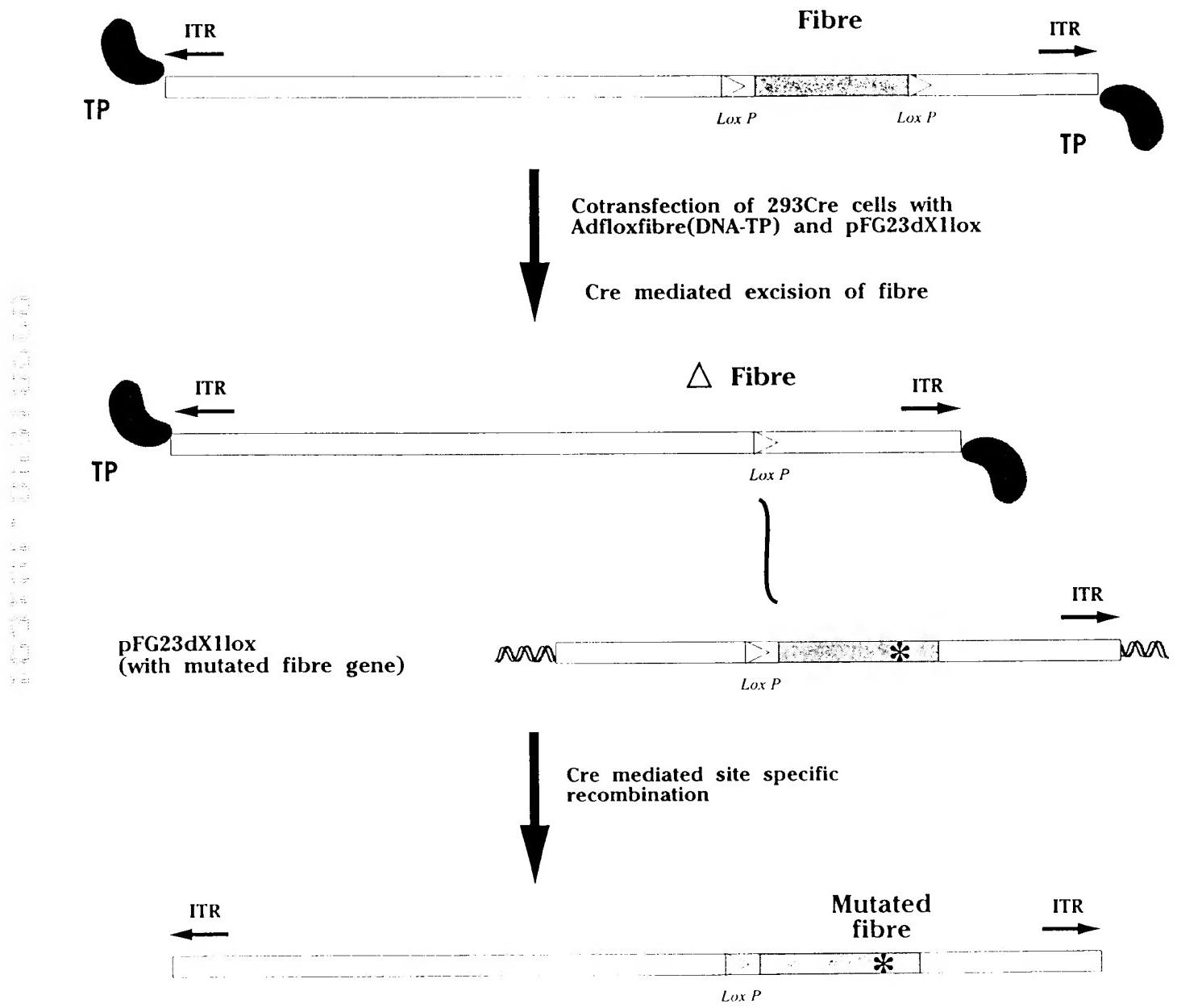


FIGURE 11B

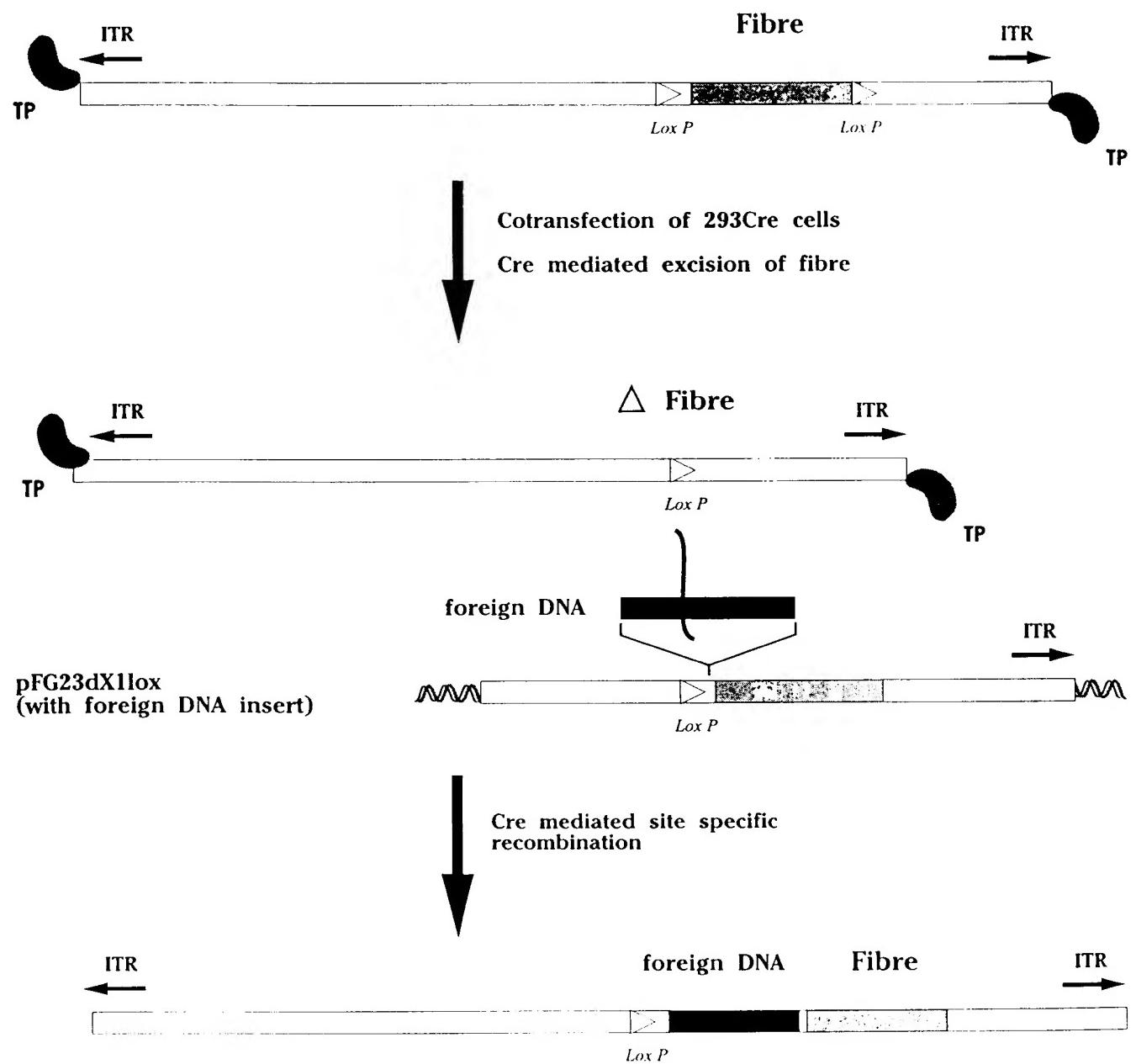
**Isolation of a virus containing a mutant fibre gene
by Cre-lox recombination using DNA-TP and cotransfection**



RECOMBINANT VIRUS CONTAINING A MUTATED FIBRE GENE

Fig. 12

Isolation of a virus containing a foreign DNA insert upstream of the fibre gene by Cre-lox recombination



RECOMBINANT VIRUS CONTAINING AN INSERT OF FOREIGN DNA
UPSTREAM OF THE FIBRE GENE

Fig. 13

CONSTRUCTION OF pAB14FL0X FOR ISOLATION OF AN AD VIRUS WITH A FLOXED FIBRE GENE

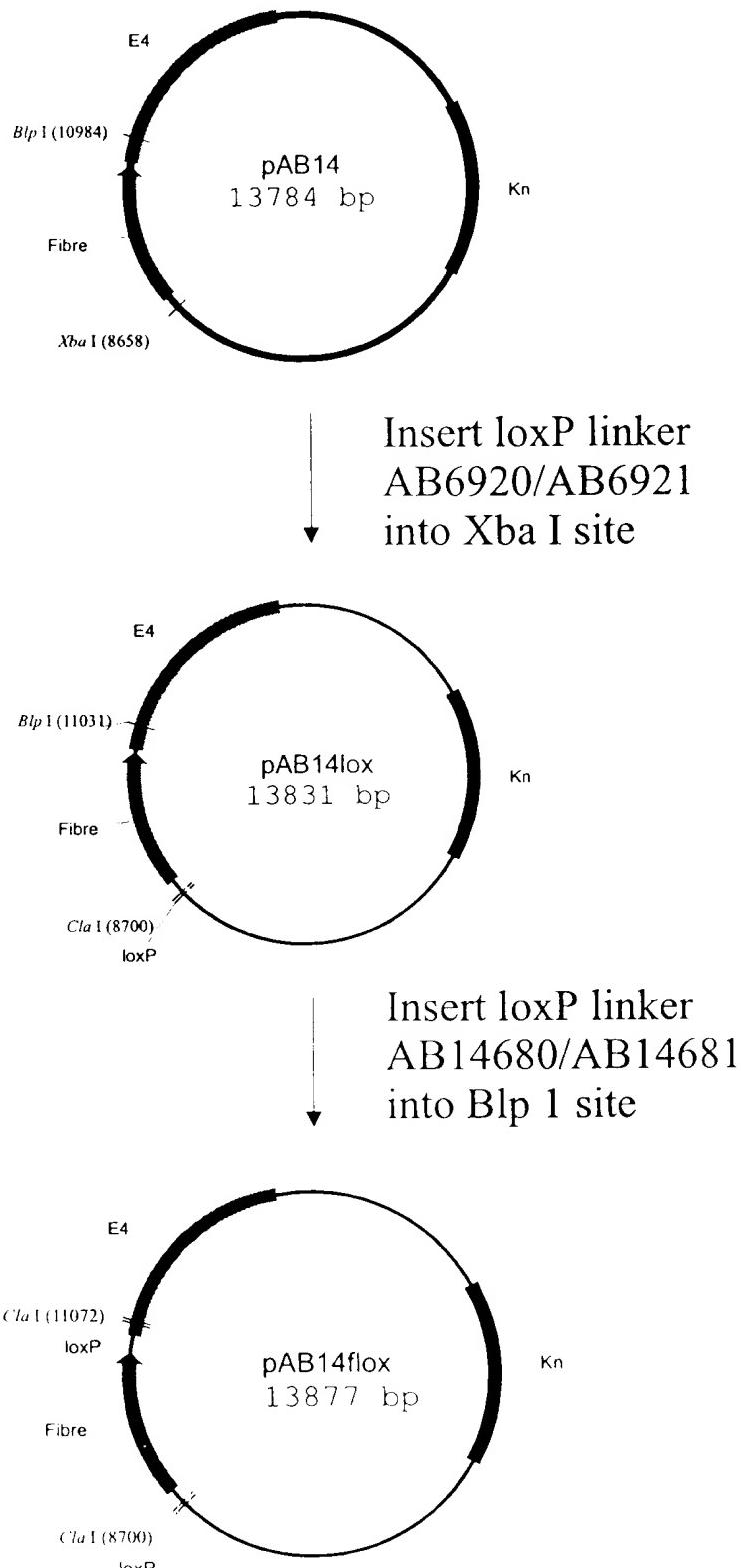


Fig. 14

Isolation of a virus containing a fibre gene with flanking lox P sites.

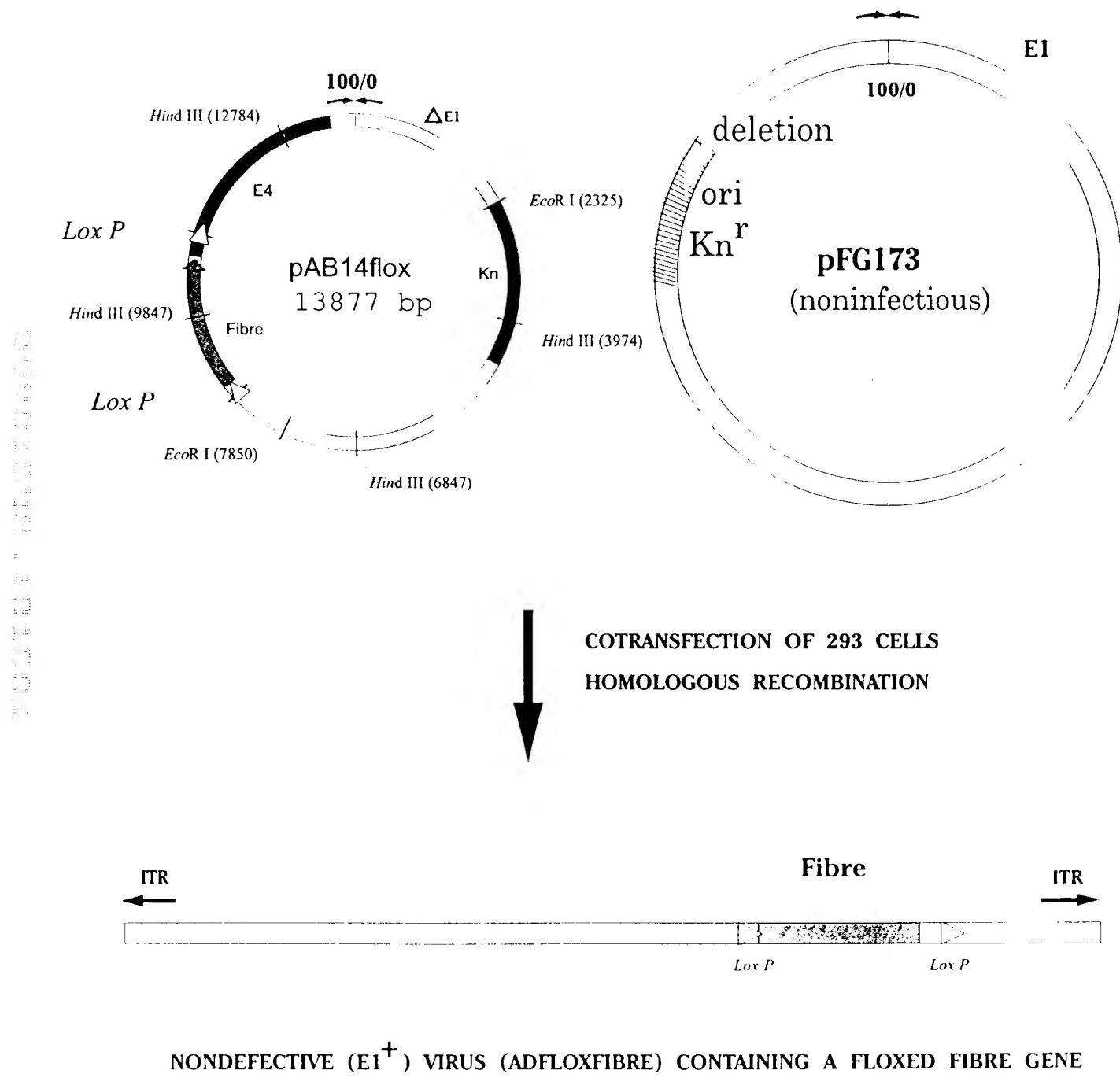


Fig.15